WHAT MAKES US TIC?

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The Prevalence of Gilles de la Tourette's Syndrome in Children and Adolescents with Autism

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Thirty-seven pupils attending a special school for children and adolescents with autism were observed for the presence of motor and vocal tics. Subsequent family interviews confirmed the diagnosis of comorbid Gilles de la Tourette's Syndrome (GTS) in three children with autism, giving a minimum prevalence rate of 8.1 %. Family history data also suggested this was heritable. The presence of GTS was not associated with superior intellectual, language, or social development. Results suggest that the rate of GTS in autism may exceed that expected by chance. The limited sample size constrains this conclusion. A large-scale epidemiological study testing this association study would appear merited.

Keywords: Autism, Tourette syndrome, comorbidity, pervasive developmental disorder.

Abbreviations: GTS: Gilles de la Tourette's Syndrome; PDD: pervasive developmental disorder; TROG: Test for Reception of Grammar.

Introduction

Gilles de la Tourette's Syndrome (GTS) is a neurodevelopmental disorder defined by the presence of chronic, multiple motor and vocal tics of childhood onset (DSM-IV: American Psychiatric Association, 1994). Typically, tics develop at 6–7 years of age, show a fluctuating course, and decrease in severity during adulthood. The precise aetiology of GTS is unknown. In most cases, GTS appears to be genetically transmitted (Curtis, Robertson, & Gurling, 1992; Eapen, Pauls, & Robertson, 1993). Implicated neurological abnormalities include functional abnormalities of the basal ganglia and prefrontal cortex (reviewed in Chase, Geoffrey, Gillespie, & Burrows, 1986), and biochemical abnormalities of the dopamine and serotonin neurotransmitter systems (reviewed in Baker, Chokka, & Bornstein, 1995). GTS is often accompanied by obsessive-compulsive behaviours (Frankel et al., 1986), which may be an alternative expression of the putative GTS gene(s) (Pauls, Towbin, Leckman, Zahner, & Cohen, 1986).

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Autism is also a neurodevelopmental disorder, itself defined by abnormal social and communication development, with a pattern of restricted and repetitive interests and activities (DSM-IV: American Psychiatric Association, 1994). Autism has an earlier age of onset than GTS, and shows a chronic and unfluctuating course. The aetiology of autism is unknown, although possible factors include genetic (Bailey et al., 1995; Folstein & Rutter, 1977), neurobiological (Bauman & Kemper, 1994), and cognitive (Baron-Cohen, 1995; Frith, 1989) abnormalities.

Although distinct disorders, autism and GTS share several behavioural features. Here we list some of these shared features, whilst pointing out how these may differ in the two syndromes: (1) Echolalia and palilalia are common in both GTS and autism, although in autism, unlike GTS, these behaviours may be appropriate to the level of speech development. (2) Types of obsessive-compulsive behaviours are frequently seen in both autism and GTS, although in autism these may be better described as rigid and ritualistic behaviour, such as an insistence upon sameness and resistance to change. (3) Like GTS, autism is associated with abnormal motor behaviours, although in autism these often take the form of stereotypies, such as spinning, rocking, and hand flapping.

Recently, a growing number of case reports have documented the co-occurrence of autism and GTS in the same individuals. Early reports described the development of motor and vocal tics in adults with autism following withdrawal from long-term neuroleptic treatment (Mueller & Aminoff, 1982; Stahl, 1980). Owing to the adult onset, Stahl interpreted the development of tics as secondary to neuroleptic withdrawal, although Mueller and Aminoff also discussed the possibility that long-term neuroleptic use may have masked or delayed the appearance of tics that may otherwise have developed spontaneously. Later case reports of childhood-onset motor and vocal tics in autism or Asperger syndrome have also interpreted their development as secondary to neuroleptic withdrawal (Littlejohns, Clarke, & Corbett, 1990; Perry, Nobler, & Campbell, 1989).

Realmuto and Main (1982) were the first to report the development of GTS in a drug-naive child with autism. These authors interpreted this co-occurrence as a chance association, as did a subsequent report of childhood-onset GTS in autism (Barabas & Matthews, 1983). However, Barabas and Matthews also discussed the possibility of a common neurochemical abnormality.

A number of case reports of GTS comorbid with autism or Asperger syndrome have since been published. Most of these later reports have speculated on possible aetiological relationships between autism spectrum disorders and GTS, including common chromosomal and genetic abnormalities (Hebebrand et al., 1994; Kerbeshian, Burd, & Martsolf, 1984a, b; Sverd, Montero, & Gurevich, 1993). This recent discussion of common aetiological factors implies a higher rate of comorbid autism and GTS than would be expected by chance. However, although the growing number of case reports suggests this higher rate, there have been few studies of the prevalence of comorbid autism and GTS.

The estimated general population prevalence of autism is 2.5 per 10,000 (DSM-IV: American Psychiatric Association, 1994). The estimated general population prevalence of GTS is 2 per 10,000 (Robertson, 1994). Hence, if autism and GTS are truly independent, the rate of co-occurrence expected by chance would be roughly 5 per 100 million.

A few studies have documented the prevalence of GTS in populations of individuals with autism spectrum disorders. Kerbeshian and Burd (1986) reported a clinical series of six individuals with Asperger Syndrome, of whom three (50%) had comorbid GTS. The same group (Burd, Fisher, Kerbeshian, & Arnold, 1987) reported a rate of 20.3% of GTS in an ascertained sample of 59 individuals meeting DSM-III criteria for infantile autism or pervasive developmental disorder (PDD). Of the 12 children with comorbid GTS, 10 had an atypical PDD, whereas only 2 had autism. Kano's group (Kano, Ohta, & Nagai, 1987; Kano, Ohta, Nagai, Yokota, & Shimizu, 1988) described 2 children with autism and GTS, drawn from a sample of 76 children with autism, suggesting a much lower prevalence rate of 2.6%.

Autism spectrum disorders may be considerably more common than autism itself. Ehlers and Gilberg (1993) estimate their prevalence to be as high as 0.74%. Fewer studies have documented the rate of autism spectrum disorders in populations of individuals with GTS. Comings and Comings (1991) reported 16 individuals with autism, Asperger syndrome, or PDD, among a clinical series of 1400 children and adults with GTS, suggesting a rate of 1.1% of autism spectrum disorders in GTS. Berthier's group (Berthier, Bayes, & Tolosa, 1993) reported 9 individuals with Asperger Syndrome among a clinical series of 100 patients with GTS, giving a prevalence rate of 9.0%.

We are not aware of any studies reporting the rate of comorbid autism and GTS in the general population. However, Sverd (1991) reported 10 children with autism or PDD and comorbid GTS. Given the population statistics for the geographical regions from which these children were clinically ascertained, Sverd argues that these children represent a rate of comorbidity exceeding that of chance.

It is clear that all reported prevalence rates exceed that expected by chance. However, two inconsistencies in the research to date require explanation. First, reported rates of GTS in autism spectrum disorders (2.6%, 20.3%, 50.0%) tend (with the exception of Kano and colleagues' statistic of 2.6%) to exceed, by far, reported rates of autism spectrum disorders in GTS (1.1%, 9.0%). These results suggest a sampling bias. One source of bias may be that studies reporting the rate of autism spectrum disorders in GTS have sampled clinic outpatient attenders. The difficulties of most children with autism are likely to be identified during the preschool years, and these children are likely to attend special schools. Unusual behaviours that develop subsequently will tend to be considered in the context of the child's autism, and as the key management of these children's needs will be met in special school placements, the need for further assessment for a subset of unusual behaviours is unlikely to be recognised or felt. Hence, outpatient samples of children with tic disorders probably under-represent children with autism, and staff at special schools for children with autism are unlikely to look for the presence of tics.

Second, the rate of Asperger syndrome in GTS appears to exceed by far that of autism in GTS (9.0% vs. 1.1%), and the rate of GTS in Asperger syndrome appears to exceed by far the rate of GTS in autism (50.0% vs. 2.6%–20.3%). (Note that Comings and Comings' figure of 1.1% includes individuals with Asperger syndrome and PDD, and that Burd and colleagues' figure of 20.3% includes individuals with atypical PDD.) This may again reflect a sampling bias. Children with Asperger syndrome, or high-functioning forms of autism, are more likely than children with autism to receive a late diagnosis and to receive mainstream schooling, and these factors may render the identification of tics, and so referral to outpatient GTS clinics, more likely.

This is important as Kerbeshian and Burd's group (Burd et al., 1987; Kerbeshian et al., 1984a, b) has suggested that the development of GTS in children with autism is a marker for improved developmental outcome. In their study (Burd et al., 1987), the comorbid group had a higher mean IQ (70.4 vs. 45.5), and superior receptive and expressive language skills, than the group with infantile autism or PDD alone. Similarly, Sverd (1988) reported two children with GTS comorbid with a mild form of PDD, and suggested that in some cases of PDD, the co-occurrence of GTS may be an indication of a less

severe variant of PDD. Contrary to these reports, Kano and colleagues (Kano et al., 1987, 1988) have reported two young adults with comorbid autism and GTS, followed since early childhood, neither of whom showed improved intellectual, language, or social functioning following the development of GTS.

The study presented here aimed to establish the rate of GTS in a special school population of children and adolescents with autism. This population was chosen in order to avoid possible sampling biases created by relying solely on outpatient clinics. In addition, the present study employed a prospective, multi-stage design, using both direct observation and family interview methods.

Method

Participants

All pupils attending a special school for children and adolescents with autism were invited to take part in the study. The total school population was 61 pupils, with a mean age of 14:2 (years:months; range 10:10 to 18:9), and a sex ratio of 3.7:1 (male:female). The parents of 41 (67.2%) children responded. Of responders, the parents of 39 (95.1%) children consented and the parents of 2 (4.9%) children declined to take part in the study. Two children for whom parental consent was given were unavailable for observation at the time of the study. Hence, the total number of children participating was 37, with a mean age of 14:2 (range 10:10 to 18:9), and a sex ratio of 4.3:1 (male:female).

Procedure

Stage 1: Case-note identification. The case-notes of all participating children were reviewed to identify any children who had previously been assessed for a tic disorder, or had previously received a diagnosis of GTS.

Stage 2: Teacher identification. The symptoms of GTS were discussed with teaching staff with the aid of a video showing motor and vocal tics in a child and adult with GTS. Teachers were subsequently asked to identify any child who had ever displayed similar behaviours at school.

Stage 3: Observation. Two observers (CM & JM) carried out independent observations of all participating children during a 2-day period. Both observers are experienced in the assessment and diagnosis of GTS. Children were observed at school, in their classrooms, and during their usual school activities. An attempt was made for each child to be observed at different times of the day (morning and afternoon) by the two observers, although this was not possible for all children. Each child was observed for at least 20 minutes (10 minutes by each observer), using a time-interval sampling observational procedure. The presence of motor and vocal tics, and of other abnormal movements, was recorded. Each child was subsequently classified by each observer independently as having motor tics, vocal tics, motor and vocal tics, or not tics, on observation. Children classified as having motor *and* vocal tics, when both observers' observations were pooled, were entered directly into Stage 5. Children classified as having only motor *or* vocal tics, when both observers' observations were pooled, were entered into Stage 4.

Stage 4: Re-observation. All children classified as having motor *or* vocal tics (but not motor *and* vocal tics) at Stage 3 were re-observed at their school by one observer (CM), for a further 20 minutes each, 4 weeks later. Children for whom both motor *and* vocal tics had been observed, when all observations (Stages 3 and 4) were pooled, were also entered into Stage 5.

Stage 5: Family interviews. The parents of the children classified as showing both motor *and* vocal tics on observation were invited to discuss their child's possible GTS. Interviews were conducted at the children's school, by a neuropsychiatrist (MMR) and psychologist (SBC) experienced in the specialist assessment and diagnosis of GTS and autism, respectively.

DSM-III-R (American Psychiatric Association, 1987) criteria for GTS were used in preference to DSM-IV (American Psychiatric Association, 1994) criteria, as DSM-IV requires the additional criterion that tic symptoms must cause marked distress or significant impairment. The adoption of this subjective criterion is inappropriate for many research purposes (Comings, 1995; Erenberg & Fahn, 1996; Freeman, Fast, & Kent, 1995). Particular issues for this study are that personal distress may not be experienced by young children, and may be difficult to establish in children with limited communication and in some children with learning disability. The interviewers also observed each (Stage 5) child on the same day as the family interviews, either as part of the interview, or during the child's usual school activities.

Subsequently, previously obtained results on the Test for Reception of Grammar (TROG) measure of vocabulary and syntax comprehension (Bishop, 1983) were made available to the research team for each Stage 5 child.

Results

The numbers of children identified at each stage of the study are shown in Table 1.

At Stage 2, the teachers of 29 of the 37 participating children were available for discussion at the time of the study. Nine of these 29 children (31.0%) were identified by their teachers as having shown a history of tic-like behaviours. This information was not used to classify children, as teachers reported some difficulty in differentiating between voluntary and involuntary movements and noises. (However, five of the nine teacher-identified children were also identified by the trained observers as showing motor and/or vocal tics, and two of these nine were later diagnosed with GTS.)

Four children entered Stage 5 of the study. The parents of one child declined to be interviewed. For the remaining three children, at least one parent was interviewed. For two of the children, a second parent or other relatives also took part in the interview. The diagnosis of GTS was confirmed after family interview for all three children. Family history data confirmed a family history of GTS in all three cases. A brief description of each diagnosed child is given below.

Table 1
Numbers of Children Identified at Each Stage of the Study[a]

	Motor tics only	Vocal tics only	Motor and vocal tics	Total	*N*
Stage 1	0	0	0	0	37
Stage 2	4	1	4	9	29
Stage 3[b]	8	0	2	10	37
Stage 4[c]	6	0	2	(8)	8
Stage 5	0	0	3	(3)	3

[a] Non-cumulative.
[b] When both observers' data were pooled.
[c] When all observations (Stages 3 and 4) were pooled.

Child 1

Child 1 was aged 16:6 at the start of the study. He displayed multiple motor and vocal tics on observation. Simple motor tics included blinking, mouth movements, head-jerking, finger-flexing, and arm extensions. Simple vocal tics included throat-clearing and high-pitched noises. More complex motor tics included lip-smacking, face-rubbing, body-tapping, head-clasping, clapping, stamping, and whole-body jumping. Complex vocal tics included exaggerated laughing and bird-like noises. Echolalia and palilalia were also observed. He did not show any other abnormal movements, such as stereotypies, on observation.

The following history was obtained on family interview. Child 1 was born by forceps delivery, and was mildly jaundiced. He had developed tics between the ages of 7 and 12 years old, and had a wide repertoire of simple and complex motor and vocal tics. He had a positive history of both echolalia and palilalia, but a negative history of coprophenomena, echopraxia, and palipraxia. His tics had shown a fluctuating course, were exacerbated in crowds, and had decreased in severity with age. The tics did not appear to cause any distress. He also showed obsessive-compulsive behaviours, including checking and forced-touching. He had a history of mild self-injurious behaviour, in the form of hand-biting. He had a positive maternal family history of both motor and vocal tics, and a positive paternal family history of tics. He fulfilled DSM-III-R criteria for GTS, of moderate severity.

Child 1 was assessed on the TROG measure of receptive language (Bishop, 1983) at age 15:0 (18 months preceding entry into the study). He performed with an age equivalent of 5:3, giving a discrepancy of 9:9.

Child 2

Child 2 was aged 14:10 at the start of the study. On observation, he displayed several simple motor tics, such as blinking, arm-extensions and abductions, and whole-body jerking, and a simple vocal tic, in the form of a loud guttural noise. He did not show any complex tics on observation, although his tics were of marked intensity. He did not show any other abnormal movements, such as stereotypies, on observation.

The following history was obtained on family interview. Child 2 had been born by forceps delivery, and had been mildly jaundiced. He had suffered several febrile convulsions between the ages of 5 and 7 years of age. He had developed motor tics at the age of 5 years in the form of blinking, and vocal tics at the age of 9 years. He had a positive history of echopraxia, but a negative history of coprophenomena, paliphenomena, and echolalia. Typically, his tics had shown a fluctuating course, had increased in severity at adolescence, and were exacerbated by excitement and distress. At times, the tics disrupted his activities and caused distress to himself and his family. He also had marked compulsive behaviours, including checking, a concern for objects to be positioned in exactly the "right" place, and forced touching. These compulsive behaviours had warranted a trial of behaviour therapy. He also had a history of self-injurious behaviours, such as smacking himself. He had a positive maternal history of motor and vocal tics. He satisfied DSM-III-R (and DSM-IV) criteria for GTS, of moderate severity. Although he had received a low dose of chlorpromazine for 1 year during adolescence, the onset of tics had preceded this treatment.

Child 2 was assessed on the TROG measure of receptive language (Bishop, 1983) at age 14:5 (5 months preceding entry into the study). He performed with an age equivalent of $< 4:0$, giving a discrepancy of $> 10:5$.

Child 3

Child 3 was aged 13:9 at the start of the study. On observation, he showed several simple motor tics, such as blinking, blowing-out, and whole-body jerking, and some simple vocal tics, such as whooping noises. He also displayed palilalia on observation. He showed additional stereotypical behaviours on observation, as well as overactivity, jumping, and a variety of noises which were not considered tic-related.

The following history was obtained on family interview. Child 3 was born by caesarean section, at 10 days post-maturity. He had developed facial grimacing between 3 and 4 years of age, and grunting at 7–8 years, and ticks had shown a fluctuating course. He had a positive history of echolalia and palilalia, but a negative history of coprophenomena, echopraxia, and palipraxia. The tics did not appear to cause any personal distress, although the onset of excessive blinking at age 11 had caused some concern to his family. He also showed compulsive behaviours, such as flicking light switches on and off, and forced touching, including a ritual of touching the corners of objects with his foot before leaving a room. He had a history of self-injurious behaviours, including head-banging. He had a positive maternal family history of vocal tics and obsessive-compulsive behaviours. He satisfied DSM-III-R criteria for GTS, of mild severity. Motor and vocal tics had preceded a small dose of thioridazine at 8 years of age.

Child 3 was assessed on the TROG measure of receptive language (Bishop, 1983) at age 13:4 (5 months preceding entry into the study). He performed with an age equivalent of $< 4:0$, giving a discrepancy of $> 9:4$.

Discussion

The observed rate of 8.1% of GTS in this special school population of children with autism far exceeds that expected by chance. This observed rate may currently be the most accurate estimate of the prevalence of GTS in autism, as previous studies have retrospectively assessed the rate of GTS in clinical series of individuals with autism, and have not used prospective multi-stage studies, using combined observational and family interview methods.

This study supports the use of prospective multi-stage designs, using independent observation and direct family interview methods to assess the prevalence of GTS in children with autism. It is important to use populations of children with autism who are not selected for the presence of GTS. That no participating child had previously received a diagnosis of GTS, or an assessment for a tic

disorder, supports the suitability of special school populations for future prevalence studies.

In accordance with Kano and colleagues, this study does not lend support to earlier suggestions that the development of GTS in children with autism is a marker for improved developmental outcome, or indicates a less severe variant of PDD. On clinical impression, the three comorbid children did not show superior intellectual, language, or social development to their non-comorbid peers. On the TROG measure of receptive language (Bishop, 1983), our children showed a developmental delay of at least 9–10 years. At the time of the study, expressive speech had not developed in one child (who used sign language), and the remaining two children used largely single words and stereotyped phrases. Given that these children had already reached adolescence, and that the onset of GTS was at 7–12 years, 5 years, and 3–4 years, respectively, these results suggest that these children's development was not significantly accelerated with the onset of GTS. However, a longitudinal group design using repeated quantitative measures would be necessary to compare accurately the rate of development of children with autism who develop GTS with those who do not.

We consider the rate of 8.1% of GTS in autism as a minimum prevalence estimate for several reasons. First, that a significant proportion of children not identified as having GTS did, however, show motor *or* vocal tics (but not both) on observation. Children with GTS show a fluctuating course of tic expression, and so it is plausible that those children showing only motor *or* vocal tics, if observed for a longer period, would have shown both motor *and* vocal tics, and so entered the final stage of the study. Indeed, this is demonstrated by the two children who were identified as showing both motor *and* vocal tics only after re-observation at Stage 4. Second, children identified by their teachers as showing tic-like behaviours, but not identified by the researchers as showing motor or vocal tics on observation, may have been identified by the researchers as showing tics if they had been observed at a different time, or for a longer period. For both of these reasons, the observed rate of 8.1% of GTS in children with autism may be an underestimate.

In the final section of this paper we consider some methodological issues important to future research. First, it is possible that children with autism also appeared to show a higher prevalence of alternative tic disorders (such as transient tic disorder, chronic motor tic disorder, and chronic vocal tic disorder). The possibility of an increased rate of other tic disorders in autism has relevance for genetic studies. This merits full investigation.

Second, we should be aware of the possibility that the high observed rate of tics in children with autism may simply reflect the difficulty in distinguishing tics from other abnormal movements and noises in this population. In particular, complex motor tics can be difficult to distinguish from stereotypies in the absence of self-reported subjective experiential information, particularly regarding the volitional nature of behaviour. Such self-report data is not easily available in children with autism. However, tics, in contrast to stereotypies, are typically short-lived, contextually inappropriate, and interrupt the flow of behaviour or speech. Future studies might develop quantitative measures using these observable parameters with which to distinguish tics and other abnormal movements in children with autism.

Third, the method used here was only tested in one (medium-sized) school for children with autism. The sample size is not sufficiently large to enable robust conclusions, but given the apparent link between autism and GTS re-emerging even in a study of this size, we would advocate that a large-scale prevalence study of such comorbidity is merited.

Fourth, there is the possibility that despite the elevated rates found here, the link between these two conditions may not reflect any genetic factor. Rather, the observed elevated rate of GTS in children with autism could simply be due to some other common aetiological factor such as a neurochemical and/or a frontostriatal abnormality. What we can conclude is that the elevated rate of GTS in autism support neither a chance co-occurrence, and nor is it a neuroleptic-induced artefact. Future work addressing the genetic vs. other biological explanations of the link between these two conditions will be important in furthering our understanding of their respective pathogeneses.

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The prevalence of Gilles de la Tourette syndrome in children and adolescents with autism: a large scale study

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ABSTRACT

Background. An earlier small-scale study of children with autism revealed that 8·1% of such patients were co-morbid for Gilles de la Tourette syndrome (GTS). The present study is a large scale test of whether this result replicates.

Method. Four hundred and forty-seven pupils from nine schools for children and adolescents with autism were screened for the presence of motor and vocal tics.

Results. Subsequent family interviews confirmed the co-morbid diagnosis of definite GTS in 19 children, giving a prevalence rate of 4·3%. A further 10 children were diagnosed with probable GTS (2·2%).

Conclusions. These results indicate that the rate of GTS in autism exceeds that expected by chance, and the combined rate (6·5%) is similar to the rates found in the smaller-scale study. Methodological considerations and alternative explanations for an increased prevalence are discussed.

INTRODUCTION

Gilles de la Tourette syndrome, or Tourette syndrome (GTS) is a neurodevelopmental disorder defined by the presence of chronic, multiple motor and vocal tics of childhood onset (American Psychiatric Association, 1987). The average age of onset is reported to be 5 years of age (Leckman *et al.* 1998). The tics show a fluctuating course and may decrease in severity during adulthood. The precise aetiology of GTS is unknown. In most cases however, GTS appears to be genetically transmitted (Curtis *et al.* 1992), although the exact pattern of inheritance is still unknown. Implicated neurological abnormalities include dysfunction of the basal ganglia and/or the prefrontal cortex (reviewed in Chase *et al.* 1986), and biochemical abnormalities of the dopamine and serotonin neurotransmitter systems (reviewed in Baker *et al.* 1995). GTS is often accompanied by obsessive–compulsive behaviours (Frankel *et al.* 1986; Eapen *et al.* 1997*a*). These may be an alternative expression of the putative GTS gene(s) (Pauls *et al.* 1986).

Autism is also a neurodevelopmental disorder, itself defined by abnormal social and communication development, with a pattern of restricted and repetitive interests and activities (American Psychiatric Association, 1994). Autism has an earlier age of onset than GTS (usually by 18 months of age) and often shows a chronic course. The precise aetiology of autism is also unknown, although possible aetiological factors include genetic (Folstein & Rutter, 1977; Bailey *et al.* 1995), neurobiological (Bauman & Kemper, 1994) and cognitive (Frith, 1989; Baron-Cohen, 1995) abnormalities.

Recently, a growing number of case-reports have documented the co-occurrence of autism and GTS in the same individuals. Realmuto &

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Main 1982) were the first to report the development of GTS in a child with autism. These authors interpreted this as a chance association, as did a subsequent report of GTS in autism (Barabas & Matthews, 1983). However, Barabas & Matthews also discussed the possibility of a common neurochemical abnormality.

The estimated general population prevalence of autism is 1 per 1000 (Baron-Cohen *et al.* 1996) and the estimated general population prevalence of GTS is 2 per 10000 (Robertson, 1994). This is likely to be an underestimate, as GTS may go undetected. Hence, if autism and GTS are truly independent, the rate of co-occurrence expected by chance would be 2 per 10 million of the general population, 1 per 1000 individuals with GTS, and 2 per 10000 individuals with autism.

A few studies have documented the prevalence of GTS in populations of individuals with autism spectrum disorders. Kerbeshian & Burd (1986) reported a clinical series of six individuals with Asperger's syndrome, of whom 3 (50·0%) had co-morbid GTS. The same group (Burd *et al.* 1987) reported a rate of 20·3% of GTS in an ascertained sample of 59 individuals meeting DSM-III criteria for infantile autism or pervasive developmental disorder (PDD). Of the 12 children with co-morbid GTS, 10 had an atypical PDD, while only two had autism. This group also showed significantly higher IQ than the group with PDD without GTS, and significantly higher measures of receptive and expressive language. Burd *et al.* believed this to be an indication that co-morbid GTS in children with autism provided a marker for improved developmental outcome. Kano *et al.* (1987, 1988) described two children with autism and GTS, drawn from a sample of 76 children with autism, suggesting a much lower prevalence of 2·6%.

We are not aware of any studies reporting the rate of co-morbid autism and GTS in the general population. However, Sverd (1991) reported 10 children with autism or PDD and co-morbid GTS. Given the population statistics for the geographical regions from which these children were clinically ascertained, Sverd argues that these children represent a rate of co-morbidity exceeding that of chance.

Although distinct disorders, autism and GTS share several behavioural features. Here we list some of these shared features, while pointing out how these may differ in the two syndromes: (1) echolalia and palilalia are common in both GTS and autism, although in autism, unlike GTS, these behaviours may be appropriate to the level of speech development; (2) types of obsessive–compulsive behaviours are frequently seen in both autism and GTS, although in autism these may be better described as rigid and ritualistic behaviours, such as an insistence upon sameness and resistance to change; (3) like GTS, autism is associated with abnormal motor behaviours, although in autism these often take the form of stereotypies, such as spinning, rocking and hand flapping.

A study carried out in a special school for children with autism (Baron-Cohen, *et al.* 1999), found that three out of the 37 pupils (8·1%) had co-morbid GTS. Previous studies had generated prevalence rates retrospectively from clinical series. A special school population of children and adolescents with autism was used, with a prospective, multi-stage design, using direct observation in the classroom, and, later, both pupil and family interviews. This may have produced a more accurate estimate of the prevalence of GTS in children with autism than previous studies. However, the sample size was small. The current study aimed to replicate the earlier study, to establish the rate of GTS in a special school population, but this time with a much larger sample.

METHOD

Participants

Thirty-three schools for children with autism from around England were invited to take part in the study. Of these, nine schools agreed to participate within the timescale of the study. We have no reason to suspect these were not representative of children with autism spectrum conditions more generally, as three schools were in the north, four were in the midlands, and two were in the Greater London area. The schools catered for a mixture of day and residential students. The total number of children with autism within these schools was 458, with a mean age of 11:1 (years:months; range 3:6 to 19:8) and a sex ratio of 4·9:1 (male:female). The children's parents were contacted, via the schools, to request their consent for them to take part in the study. The parents of two children

(0·4%) declined to take part. Nine children for whom parental consent was given were unavailable for observation at the time of the study. Hence the total number of children participating was 447, with a mean age of 11:1 (years:months; range 4:2 to 19:8), and a sex ratio of 5:1. The children's diagnoses showed varying degrees of autism: 280 were diagnosed as having autism; 141 received a diagnosis of autism spectrum condition; and 26 were diagnosed as having Asperger's syndrome. These diagnoses were not made by our team but were taken from the school notes, in all cases from reports by a child psychiatrist or paediatrician.

Procedure

Stage 1

The records of all children included in the study were reviewed to identify any children who had previously been assessed for a tic disorder, or had previously received a diagnosis of GTS.

Stage 2

Where possible, the symptoms of GTS were discussed with teaching staff, using an advisory leaflet adapted from the Tourette Syndrome Association, UK. Some teachers were then asked to comment on the habits and movements of children in their classes.

Stage 3

An observer (V.S., a psychologist) carried out observations of all participating children. Children were observed at school, in their classrooms and during their usual school activities. Class sizes ranged from three children to nine, with an average class size of six. Each child was observed for at least 10 min, using a time-interval sampling observational procedure. The presence of motor and vocal tics was recorded. Each child was subsequently classified as having motor tics, motor and vocal tics, or no tics, on observation. Children classified as having motor and vocal tics were entered directly into Stage 6. Children classified as having only motor or vocal tics were entered into Stage 4.

Stage 4

Children classified as having motor or vocal tics (but not motor and vocal tics) at Stage 3 were re-observed at their school by the observer, for a further 10 mins each, a few days later. Children for whom both motor and vocal tics had been observed, when all observations (Stages 3, 4 and 5) were pooled, were also entered into Stage 6.

Stage 5

Some children ($N = 55$) were later observed by an independant observer (H.H., also a psychologist) for 10 mins each. Of these children, 23 had been classified by the first observer as showing tics, and 32 had been classified as showing no tics. The names given to this observer were presented in a random order, with no indication of their initial tic classification.

Stage 6

The parents of the children classified as showing both motor and vocal tics on observation were invited to meet a psychiatrist (M.M.R. or J.I.) to discuss their child's possible diagnosis of GTS, and to obtain a family history. Children and parents were interviewed using a short version of the National Hospital Interview Schedule (NHIS) (Robertson & Eapen, 1996). The Yale Global Tic Severity Scale (YGTSS) (Leckman *et al.* 1989) was also used. The diagnostic interviews were conducted at the children's schools, or at University College London Middlesex Hospital ($N = 1$). Interviews were conducted at the child's school as far as was possible, to reduce anxiety. Each child was also observed for 30–45 min, as part of the interview. DSM-III-R (APA, 1987) criteria for GTS were used in preference to DSM-IV (APA, 1994) criteria, as DSM-IV requires the additional criterion that tic symptoms must cause marked distress or significant impairment. The adoption of this subjective criterion is inappropriate for many research purposes (Freeman *et al.* 1995; Erenberg & Fahn, 1996; Kurlan *et al.* 1997). A particular issue for this study is that impairment and distress may be difficult to establish in children with limited communication and in some children with learning disability.

If the parents were unable to attend the interview, but wished their child to be included in this stage of the study, the child was accompanied by his or her keyworker from the school, and a phone interview with the parents was conducted later by a psychiatrist. It should be noted that in a study of this kind there is a risk that stereotypies will be confused as tics.

The distinction between these is hard to draw, but if either of the raters or clinicians thought a behaviour could be a stereotypy, this was discounted as a tic, in order to err on the side of being conservative.

RESULTS

Stage 1 – review of notes

No evidence for previous assessments or diagnoses of GTS or alternative tic disorders was found in the records of any of the participating children.

Stage 2 – teacher discussion

Our previous study (Baron-Cohen *et al.* 1999) showed teachers' reports to be fairly reliable. Therefore, the children identified by their teachers as showing tic-like behaviours were noted and this data was used to help classify children.

Stage 3 – initial observation (V.S.)

Thirty children were identified as having motor and vocal tics by the first observer (V.S.) after Stage 3. These children were entered directly into Stage 6. A further 114 children were identified for whom motor or vocal tics were observed by the first observer. These children were entered into Stage 4.

Stage 4 – re-observation (V.S.)

Of the 114 children who were entered into Stage 4, 18 were unavailable for re-observation by the first observer. Their classification therefore remained the same. From the remaining 96 children, a further 10 were identified for whom both motor and vocal tics had been observed, when both Stage 3 and 4 observations were pooled. These children were also entered into Stage 6.

Stage 5 – re-observation (H.H)

Fifty-five children were seen by the second observer (H.H.), 1 to 2 weeks after the initial observations. All observations, from Stages 3, 4 and 5, were pooled (for example, if one observer saw motor tics and the other vocal tics, that child was classified as showing motor and vocal tics). Agreement on tics *v.* no tics between the two observers was 70·4%. The two observers agreed that 12 out of the 55 children were showing tic-like behaviours, 26 showed no tic-like behaviours. They disagreed on the classification of 16 children. Both observers were trained in the identification of tics by MMR at the Tourette Clinic, at the National Hospital for Neurology and Neurosurgery, Queen Square, London. The implications of this result are discussed below. When the observations of both observers were combined, the final results were as follows: out of the 447 children entered into the study, 43 children were identified as showing both motor and vocal tics, 98 were identified as showing motor tics only, and 11 were identified as showing vocal tics only.

Stage 6

Forty-three children entered Stage 6 of the study. The parents of 10 children declined to be interviewed. One child was unavailable at the time of the appointment. For the remaining children, at least one parent was interviewed. Twenty-three children were accompanied by their parents to interview, nine were accompanied by their keyworker or teacher, and the parents later interviewed by phone. The diagnosis of definite GTS was confirmed after family interview for 19 of the 32 children. A further 10 children were diagnosed as having probable GTS. If the child showed symptoms at interview and there was a personal history of symptoms, a diagnosis of definite GTS was made. If one of these two criteria was met, but not the other, a diagnosis of probable GTS was made. For the remaining children, two were diagnosed with chronic motor tic disorder, and one was diagnosed as having Rett syndrome. This is a pervasive developmental disorder, characterized by the development of autism, dementia, apraxia of gait and stereotyped use of the hands, following a period of at least 5 months of normal functioning after birth (Hagberg *et al.* 1983).

For definite cases, the rate of true positives was 59·38%, although if we include probable GTS cases, the true positive rate rises to 90·63%. The rate of false positives was 9·38%. The method employed in this study does not allow the calculation of true and false negatives.

Medication information was provided by the parents, and six of the 32 children were taking psychotropic medication (four definite, two probable GTS), three were taking anti-

Table 1. *Diagnosis, severity of tics, and family history*

Child	Diagnosis 1	Fam. history TS/Tics/OCB	Yale score (%)	Diagnosis 2
1	Autism	M	6	Mild GTS
2	Autism	x	15	Probable GTS
3	Autism	P	15	Mild GTS
4	Spectrum	x	17	Probable GTS*
5	Autism	M	18	Definite CMT
6	Autism	P	48	Mild GTS
7	Spectrum	M	17	Definite GTS
8	Autism	x	40	Probable GTS
9	Spectrum	x	26	Probable GTS
10	Autism	P	41	Severe GTS
11	Autism	M	54	Mild GTS
12	Autism	?M	40	Severe GTS
13	Autism	M	24	Mild GTS
14	Autism	P	49	Mild GTS
15	Spectrum	M	62	Mild GTS
16	Autism	x	12	Mild GTS
17	Autism	x	35	Probable GTS
18	Autism	PM	7	Prob. CMT*
19	Spectrum	x	16	Mild GTS
20	Autism	P	30	Mild GTS
21	Spectrum	M	21	Mild GTS
22	Autism	PM	4	Probable GTS*
23	AS	P	56	Moderate GTS
24	Autism	PM	7	Definite CMT
25	Autism	P	33	Mild GTS
26	Autism	M	37	Mild GTS
27	Spectrum	M	25	Probable GTS
28	Autism	M	25	Mild GTS
29	Autism	P	32	Probable GTS
30	Autism	M	15	Mild GTS
31	Autism	PM	63	Probable GTS
32	AS	M	18	Mild GTS

* Child 4, probable GTS, definite chronic motor tic disorder (CMT); Child 18, probable CMT, definite Rett's syndrome; Child 22, probable GTS, definite chronic vocal tic disorder.

Family history: Tourette syndrome/tics/obsessive–compulsive behaviours; M, history on maternal side of family; P, history on paternal side of family; x, no family history.

AS, Asperger's syndrome.

convulsants (two probable GTS, one Rett syndrome plus probable chronic motor tic disorder) and one was taking ritalin (definite GTS). The onset of tics in this child predated the commencement of medication.

YGTSS scores were calculated for all 32 children. The scores ranged from 4% to 63%, with a mean of 28% (S.D. = 16·7).

For the children found to have co-morbid GTS and autism, 20 had been diagnosed with autism (7·1% of children with autism had co-morbid GTS), seven had been diagnosed with an autism spectrum condition (5·0% of children with an autism spectrum condition had co-morbid GTS) and two had been diagnosed with Asperger's syndrome (7·7% of children with Asperger syndrome had co-morbid GTS). When the frequencies of co-morbid GTS in children with autism, autism spectrum conditions and Asperger's syndrome were compared, there were no significant differences ($\chi^2 = 0{\cdot}43$, df = 2, NS).

Family histories were collected for all 32 children. For 25 of the 32 children (78%), there was found to be a paternal and/or maternal family history of tics and/or obsessive–compulsive behaviours. These results are shown in Table 1.

DISCUSSION

The observed rate of 6·5% of GTS (including 'probable' GTS cases) in this special school population of children with autism far exceeds that expected by chance. This study, with its prospective, multi-stage design, using combined observational and family interview/history methods, and large sample size, is likely to have

yielded the most accurate estimate yet of the prevalence of GTS in children with autism. Previous studies have retrospectively assessed the rate of GTS in clinical series of individuals with autism, and the earlier study carried out by this team (Baron-Cohen *et al.* 1999) had a much smaller sample size, although it used a similar method. The rates found in these two studies are also similar ($\chi = 0{\cdot}43$, df = 1, NS). Thus, the current study supports the findings of the smaller study, and the methods used.

It was suggested (Burd *et al.* 1987) that co-morbid GTS in children with autistic-type conditions provides a marker for improved developmental outcome. The present study was unable to collect data on IQ, and measures of receptive and expressive language, and so cannot be directly compared with Burd *et al.*'s study. However, the fact that GTS was found to be equally common in children with autism, children with autistic spectrum conditions and children with Asperger's syndrome shows that GTS is not related to the severity of autism in the child.

It is also notable that a significant proportion of children not identified as having GTS did, however, show motor or vocal tics (but not both motor and vocal tics) on observation. One hundred and nine children (24·4%) were showing tics on observation, but did not show full GTS symptoms. Children with GTS show a fluctuating course of tic expression, and so it is plausible that some of those children showing only motor or vocal tics, if observed for a longer period, would have shown both motor and vocal tics, and so would have entered the final stage of the study. Indeed, this is demonstrated by the 10 children who were identified as having both motor and vocal tics only after re-observation at Stage 4. This is also evident in the four children who were identified as having only motor or vocal tics, or even no tics in one case, by one observer, but as having both motor and vocal tics by the other, either a week later or a week earlier, and the one child who was observed as having motor tics only by one observer, and vocal tics only by the other. This may point to the need for a longer period of observation, to increase the chances of identifying those children with GTS. The observed rate of 6·5% of GTS in children with autism may, therefore, be an underestimate.

The high rate of tics observed in these children (34·0% of children were classified as showing tics)[1] is interesting, as no children had previously been diagnosed as having a tic disorder. This may indicate that children with autism also show a higher prevalence of alternative tic disorder, chronic motor tic disorder and chronic vocal tic disorder, but that this is being overlooked, possibly since they are occurring in the context of the other problems associated with autism. The possibility of an increased rate of other tic disorders in autism has relevance for genetic studies.

Is it also possible that the high observed rate of tics in children with autism reflects the difficulty in distinguishing tics from other abnormal movements and noises in this population? This differential diagnosis problem has been previously documented (Burd *et al.* 1987). In particular, complex motor tics can be difficult to distinguish from stereotypies in the absence of self-reported subjective experiential information, particularly regarding the volitional nature of behaviours. Differentiating vocal tics from the wide range of vocal productions in children with autism may be even more problematical. Apart from the clear-cut tics and stereotypies, there are quite a number of behaviours that can only be understood through careful enquiry about the nature of the movement or noise, its longitudinal course, and possible alternative explanation for the symptom. For example, a vocalization, which was initially noted as a sniffing tic, was later discovered, after questioning, to be a repetitive imitation of a TV character with which the child was currently obsessed. However, as mentioned in the Burd *et al.* study, tics, in contrast to stereotypies, are typically short-lived, contextually inappropriate and interrupt the flow of behaviour or speech. It was also attempted to systematize the difference between motor tics and stereotypies by their topography (tics tend to be clearer in the face, neck shoulders and arms, compared with hands and fingers), their nature (spasmodic *versus*

[1] This prevalence includes those children: diagnosed with probable or definite, GTS (29) and CMT (3); with parents who declined to be interviewed in Stage 6 (10) and one child unavailable for diagnosis (these 11 children were classified as either motor or vocal, as their tics had not been verified by a psychiatrist); who were showing motor or vocal tics on observation by V.S. and/or H.M. (109). Therefore, the total number of children showing tics was 152.

rhythmic) and also by the quality of the subjective experience and their response to psychosocial factors (Kano *et al.* 1988). These factors were taken into account when the observations were made, and we are therefore confident that the result obtained represents true co-morbidity.

It is well-known that the tics encountered in GTS wax and wane in severity and fluctuate with time. Stress or anxiety may increase tics, whereas concentrating on a task may reduce tics. Thus, if the raters observed the children with autism at different times, for up to 10 min, and on different days (sometimes more than a week apart), it is quite conceivable that different tics occurred at different times. The levels of stress and anxiety may also have been different, and the children may also have been observed at different times during different tasks (e.g. concentrating). The raters were trained at the Tourette clinic at the National Hospital Queen Square during both new-patient and follow-up clinics. Inter-rater reliability was assessed on the tic observation section of the NHIS. Training was carried out for approximately 6 weeks, seeing about 40 GTS patients (V.S.), while the other psychologist (H.H.) sat in on the clinic and rated many more GTS patients over a year's duration. Agreement between the two raters was only modest, and this may well have been due to factors described above.

A study by Eapen *et al.* (1997*b*) found that children in a special school population had an increased prevalence of GTS. Fifty-five per cent of emotionally/behaviourally disturbed children (EBD) and 20% of children classed as learning disabled (LD) were diagnosed as having GTS. Whereas the EBD result could indicate that children with EBD have such problems due to the disruptive effects that GTS can have on the individual, the LD result could point to a larger picture – that children with learning difficulties are more prone to GTS. IQ data were not available for the children in the current study, thus we cannot say whether the results we obtained, of 6·5% GTS in children with autism, can be attributed to their more general learning difficulties. Further research in this area is necessary to better clarify the links between these conditions.

The generally accepted prevalence figure for GTS is around 0·5 per 1000 (Bruun, 1984); this figure has also been reached in a careful epidemiological study (Apter *et al.* 1993). A more recent pilot study by Banerjee *et al.* (1998) in the UK yielded higher results and found the rate of GTS in a mainstream school population to be around 3%. This study, however, had a small sample size ($N = 166$), and the identified cases were not re-assessed and formally diagnosed by an expert. It has been criticized (Traverse, 1998) and stirred debate (Banerjee *et al.* 1998). The actual prevalence rate of GTS has, therefore, yet to be determined, but the currently accepted figure remains around 0·5 per 1000. We await the results of our larger definitive study in a UK school population.

The YGTSS scores in our GTS individuals ranged from 4% to 63% with a mean of 29% (see Table 1). The majority of the scores indicate mild to moderate severity. The scale range is 0–100%. In the only UK GTS clinic study published using the scale (a modified version with the total range being 0–55) the GTS cases scored a mean of 26·2 (range 11–55) (Robertson *et al.* 1997). This would be between 45% and 55% using the currently used version of the scale. In another study (Robertson *et al.* 1999), on 280 consecutive GTS clinic cases, the YGTSS scores ranged from 1 to 100% (mean 49%; S.D. = 23). Both these studies indicate that the GTS/autism individuals are not nearly as severe as clinic patients. In the present study, some of the YGTSS global tic severity scores may have been higher than expected in this population due to the presence of, for example, echophenomena, which occur in both GTS and autism and which symptom receives a separate score on the YGTSS.

The short version of the NHIS (Robertson & Eapen, 1996) was used in this study. This goes into detail with regard to individual tics, and the interviewer records whether specific tics have taken place in the past (ever), in the past week (which allows the YGTSS to be completed), as well as those observed at interview. Tics include simple ones such as frowning, raising eyebrows, blinking, winking, eye movements, nasal twitches, mouth twitching, pouting and opening, tongue protrusion, facial grimacing, platysma tightening, head nodding and shaking, shoulder shrugging and flicking the hair out of the eyes; simple vocalizations include grunting throat clearing, sniffing, snorting, grunting and

coughing. These were the majority of tics noted in the study. Complex tics (motor and vocal) and stereotypies may, however, be difficult to differentiate from each other, and include, for example, hand-flapping (common in people with autism; rare in people with GTS, and not regarded as a tic in this study), twirling (also not encountered in this study), vocalizations, inappropriate fluctuations in pitch of the voice, and squealing. Of course, some symptoms such as echolalia and echopraxia, are common in both autism and GTS, and may be indistinguishable from each other phenomenologically in each condition. It is acknowledged that the relatively higher scores in the complexity score of the YGTSS may well have been partly due to the presence of symptoms such as ecophenomena; one author (M.M.R.) rated the pupils on the YGTSS and included only what she considered to be tics, apart from the ecophenomena. It must be noted that 18 pupils had echolalia, while 12 had echophraxia (10 of these had echolalia as well). We acknowledge the difficulties in differentiating between the two.

Our new screening method suggests that a larger scale cognitive study of the effects of co-morbidity would now be possible. These effects were investigated in the Baron-Cohen and Robertson (1995) case studies. A child with autism, a child with GTS and a child with both conditions were tested for theory of mind, intention-editing and executive function deficits. The predictions, that the child with autism would show a deficit in theory of mind tests, that the child with GTS would show a deficit in intention-editing tests, and that the child with both conditions would show deficits in both these areas, were supported. A larger scale study, with similar methods, may help to confirm these results, which have the natural limitations of single case studies.

One of the purposes of this study was to find children who were being overlooked for treatment. In co-morbid cases of autism and GTS, the latter is often overlooked (as in 100% of our cases) and the symptoms are, therefore, left untreated. Finding these co-morbid cases allows the children's pharmacological management to be reviewed, and alleviation of tics can help to improve their quality of life. Three such cases were found in this study. By themselves, these cases provide a clinical justification for such screening. For the other children, diagnosed with mild GTS, and not needing medication, knowledge of their condition can help parents and teachers in their continuing support for the child.

The observed elevated rate of GTS in children with autism is consistent with the operation of common aetiological factors, and does not support a chance co-occurrence. Possible common aetiological factors include neurochemical and frontal lobe abnormalities. That there is a substantial family history of GTS or GTS spectrum disorders suggests that there may also be independent genetic mechanisms at play. Future work addressing these possibilities will be important in furthering our understanding of the respective pathogeneses of these two neurodevelopmental disorders.

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Diagnosing Tourette syndrome Is it a common disorder?

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Abstract

Objectives: The evaluate the prevalence of Tourette syndrome (TS). **Methods:** A review of the literature on TS was undertaken to examine the prevalence of TS in mainstream children as well as those in special education. **Results:** Recent studies have indicated that TS occurs in around 1% of youngsters in mainstream schools between the ages of 5 and 16 years. It is even more common in youngsters with special educational needs. **Conclusions:** TS is more common than was previously documented.

Introduction

Tourette syndrome (TS) is an inherited neuropsychiatric disorder characterised by the presence of both multiple motor tics and one or more vocal tics that last for longer than a year and begin in childhood or early adolescence [1,2].

Diagnostic criteria

The diagnostic criteria for diagnosing TS will be briefly considered in the article, and it will be shown how the systems for diagnosing TS have evolved and indeed been controversial at times. For instance, the WHO system has remained fairly static and has had no age limit, while the DSM criteria of the APA have stipulated upper age limits that have, in fact, changed with time. In the DSM-IV-TR [1] current diagnostic criteria, the onset must be before the age of 18 years. In addition, DSM-IV [3] was controversial, as evidenced by several letters to the editor challenging, inter alia, the stipulations of impairment and distress in order to make the diagnosis [4–7]. In addition, the Tourette Syndrome Classification Study Group [8] suggested that, by and large, the DSM-III-R criteria [9] were those seen as acceptable in TS research.

It is worth remembering, at this juncture and from an historical perspective, that the syndrome that was named after Georges Gilles de la Tourette [10], consisted of multiple motor tics, coprolalia, echolalia, and motor incoordination. How many TS cases seen in general and expert clinics and also especially epidemiological, prevalence, and genetic studies today do actually fill these particular criteria? It is suggested that it is the small minority.

Epidemiology and demography

TS used to be considered a very rare condition, with only case reports documented in the literature [11,12] and a small international registry published [13]. Throughout the 1980s and the 1990s, however, the medical literature on TS mushroomed, and many large cohorts of TS patients were described, including several dedicated prevalence studies.

The prevalence of tic disorders has been the subject of study for some time, and the generally agreed prevalence figures range from 4% to 18%, with the majority of studies finding that about 10% of youngsters have tics (for full review, see Ref. [14]).

TS is a category with in the tic spectrum and is one of the manifestations of childhood tics. The generally accepted prevalence for TS has always been much less common than

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for tics and for some time was thought to be 5/10,000 [15]. Recently, it has been demonstrated that TS is much more common than previously suggested as evidenced by two pilot studies [16,17] and four definitive investigations embracing a relatively large number of youngsters [18–21]. For a summary of the prevalence studies in mainstream school populations, see Table 1. It should be noted that the senior authors in all these studies all have a special interest in TS, and that the methods used included direct observations of the youngsters under study. It is thus suggested that a more realistic figure for the prevalence of TS is around 1% of school children between the ages of 6 and 17 years old.

The prevalence of TS in children with special educational needs [16,22,23] and autistic spectrum disorders [24,25] is, however, even higher than that in normal mainstream children.

As psychopathology and comorbid disorders are commonly associated with TS [11,26], it was decided to assess the prevalence of tics and TS in a psychiatric inpatient population in a cross-sectional study using semi-structured interviews [27]. None of the 200 patients examined had definite TS, but two were observed to have motor tics at interview and 10 had a history of tics (present for less than a year). The results did not support the theory that TS is overrepresented among adult inpatients with psychiatric illness.

TS is found in all cultures, countries, and racial groups and occurs three to four times more commonly in males [11,12,26].

Clinical phenomenology

It may be clinically useful to subdivide TS into three groups [28]. (i) The first is "simple" TS (with motor and vocal tics being the predominant and almost the only symptoms). (ii) Secondly, there may be "full blown TS" (with coprophenomena, echophenomena, and paliphenomena. (iii) Thirdly, there may be "TS plus" in which the TS patients may also have attention deficit hyperactivity disorder (ADHD), obsessive–compulsive behaviours (OCB), self-injurious behaviours (SIB), and a variety of kinds of complex psychopathologies.

In the majority of studies, TS seems to start at the mean age of 6.7 years (range 1–17), with motor tics (such as eye blinking), followed by the vocal tics around the age of 9 (such as throat clearing and sniffing) [29]. Coprolalia (which is seen in about 30% of clinic TS patients and 10% of all TS individuals) begins at around the age of 15 [12]. Goetz et al. [30] studied 58 adult TS patients who had been diagnosed during childhood. It was reported that tics persisted in all patients but were moderate/severe in only 24% when compared to 60% at the lifetime point of worst function. Coprolalia persisted in 4% compared with 22% during the period of worst function. For most TS patients, worst function had occurred during adolescence (mode = 13 years). Of importance is that despite a high frequency of school and behavioural problems during development, the vast majority (90–98%) had completed high school and were either full-time students or in employment [30]. A similar and more recent study has suggested a slightly earlier age of onset of TS (5.6 years), severe tics at around 10 years, and the majority of symptoms disappearing in half of the patients by the age of 18 years [31]. Thus, the prognosis of TS is better than previously thought.

Investigations have also demonstrated that TS clinic patients also have significant nonobscene complex socially inappropriate behaviours (NOSI) [32], plus a variety of psychopathologies [33], but it is unclear whether or not these behaviours and psychopathologies would be found in TS cases identified in the community. In one large multi-country study [26], only 12% of TS patients had no other psychopathology or comorbidity.

Psychopathology and comorbid disorders

Suggested common associations in clinical TS cohorts include ADHD and obsessive–compulsive disorder (OCD). The prevalence of ADHD is about 5% in the general population and that of OCD is between 1.9% and 3.2% [14]. In this context, there has been a suggestion [34] that if one were to examine the suggested TS spectrum, i.e., tics, TS, ADHD, and OCD, the prevalence would be about 10% of the population, which is clearly far from uncommon.

Table 1
Prevalence of TS in mainstream school populations (after Lanzi et al. [21])

Author	Year	Country	Age (years)	Sample size	Procedure	Prevalence (%)
Kurlan et al. [16]	1994	USA	7–14	35	observation + interview	3.0
Mason et al. [17]	1998	UK	13–14	166	self-report, parents' Q, teachers' Q, and observation	2.9
Kadesjo and Gillberg [19]	2000	Sweden	11	435	clinical examination	0.15–1.1
Hornsey et al. [18]	2001	UK	13–14	918	self-report, parents' Q, teachers' Q, and two interviews	0.76–1.85
Kurlan et al. [20]	2001	USA	8.5–17.5	1255	interviews	3.8
Lanzi et al. [21]	2002	Italy	6–11	2347	classroom and observation	0.68

After Lanzi et al. 2002 [21].

Aetiology

It is now generally recognised that TS is a genetic disorder with most evidence suggesting a single major gene locus with suggestions of autosomal dominant inheritance, although more recently other mechanisms of inheritance have been suggested [33], but to date no single gene has been identified. There may be several reasons for this nondetection of gene(s), including clinical heterogeneity, consequent incorrect definition of the phenotype and genetic heterogeneity (i.e., different gene(s) in different families). The recent first genome scan [35] identified areas of interest on chromosomes 4 and 8. However, other factors have been very recently invoked in the aetiopathogenesis of TS and have included streptococcal infections in some patients [36–38] and pre- or perinatal events [39].

It is suggested that only when the TS gene(s) are found, will it be clearer as to which of the above aetiological and genetic explanations are correct, and in fact, what constitutes the precise TS phenotype. Whether or not this phenotype includes only tic phenomena, or whether it includes behaviours and psychopathology as well will be clearer. Only then will the true prevalence of both tics and TS be known.

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THE AETIOLOGY OF

GILLES DE LA TOURETTE SYNDROME

Genetics

Neuroimmunology

GENETICS

British Journal of Psychiatry (1990), 156, 515-521

Familial Tourette's Syndrome in a Large British Pedigree
Associated Psychopathology, Severity, and Potential for Linkage Analysis

MARY M. ROBERTSON and ALISON GOURDIE

A British pedigree multiply affected by the Gilles de la Tourette syndrome and spanning six generations is described. Of 122 members identified, 85 were individually interviewed, and 50 were diagnosed as 'cases'. 'Cases' consisted of 29 with definite or probable Gilles de la Tourette syndrome, 17 with definite or probable chronic multiple tics, and four with definite or probable obsessive-compulsive behaviour. Only eight of the 50 'cases' had consulted a doctor for their symptoms. 'Cases' and 'non-cases' could be distinguished on the basis of echo-phenomena, obsessive-compulsive features, self-injurious behaviour, and the trait score of the Leyton Obsessional Inventory, but did not differ significantly on any other psychopathological variables. The pattern of inheritance of the Gilles de la Tourette syndrome in this pedigree is consistent with autosomal dominant transmission.

The precise aetiology of the Gilles de la Tourette syndrome (GTS) is still unknown. In 1885, George Gilles de la Tourette postulated the hereditary nature of the disorder. Many investigators have subsequently demonstrated that GTS and chronic multiple tics (CMT) show a familial pattern (Eldridge *et al*, 1977; Golden, 1978; Guggenheim, 1979; Kidd *et al*, 1980; Nee *et al*, 1980; Pauls *et al*, 1981; Jagger *et al*, 1982; Erenberg *et al*, 1986; Lacey, 1986). Moreover, data from studies involving 11 concordant monozygotic GTS sets of twins suggest a genetic influence in GTS (Escalar *et al*, 1972; Wasman *et al*, 1978; Shapiro *et al*, 1978; Jenkins & Ashby, 1983; Waserman *et al*, 1983; Vieregge, 1987) and CMT (Price *et al*, 1985). In the latter study 43 pairs of same-sex twins were studied in which at least one co-twin had GTS. Concordance rates for GTS were 53% for monozygotic pairs and 8% for dizygotic pairs, but when diagnostic criteria were broadened to include tics in a co-twin, concordance rates rose to 77% and 23% for monozygotic and dizygotic twins respectively. It has been suggested that in families with GTS, CMT may be a milder manifestation of the disease (Pauls *et al*, 1981). A study of one large Mennonite extended family, of whom 159 were interviewed, suggests the transmission of GTS and CMT is autosomal dominant (Kurlan *et al*, 1986, 1987).

Recent psychopathological studies suggest that obsessive-compulsive behaviour (OCB) is not only severe in many patients with GTS (Frankel *et al*, 1986), but may be an integral part of the disorder, as it is significantly associated with central features of the syndrome such as copro- and echo-phenomena (Robertson *et al*, 1988). This link between GTS and OCB does not appear to be culturally determined, as OCB has been reported as occurring in significant proportions of American GTS cohorts (Comings & Comings, 1987*a*,*b*), and in equivalent cross-cultural American-British cohorts (Frankel *et al*, 1986), and in both British (Robertson *et al*, 1988) and Dutch (van der Wetering *et al*, 1988) GTS populations. Moreover, recent studies have suggested that GTS and OCB may be aetiologically and even genetically related (Pauls *et al*, 1986*a*,*b*).

Initially identified through the index case who presented at the clinic at the National Hospital for Nervous Diseases, Queen Square, we have identified and studied a large British kindred spanning six generations, and including 122 members plus spouses. In this paper we report the clinical and psychopathological features in the members of this extended family.

Method

We evaluated individuals in the pedigree over a two-year period. Initially, the subjects were interviewed to assess 'caseness', using a short, semistructured interview, lasting approximately 15-20 minutes, and devised by one of us (MMR) who is well acquainted with the clinical presentation and diagnosis of GTS. 'Caseness' was defined as the presence of symptoms or history sufficient to fulfil the criteria of one of the five diagnostic categories (see below). In addition, each individual over 18 years-of-age was assessed using the Schedule for Affective Disorders and Schizophrenia, Lifetime version (SADS-L; Endicott, 1978). The adult members of the pedigree (over 18 years) were also asked to complete the following standardised

psychiatric rating scales to assess specific aspects of psychopathology: the questionnaire form of the Leyton Obsessional Inventory (Snowdon, 1980); the Crown-Crisp Experiential Index (CCEI), previously known as the Middlesex Hospital Questionnaire (Crown & Crisp, 1966); the Hostility and Direction of Hostility Questionnaire (HDHQ; Caine *et al*, 1967); and the 30-item version of the General Health Questionnaire (GHQ; Goldberg, 1972). These particular rating scales were chosen as it has been reported that patients with GTS referred to a clinic not only have high general psychopathology, but that both obsessionality and hostility are intimately linked to central features of GTS (Robertson *et al*, 1988).

Fifty-eight pedigree members were reinterviewed, using a more detailed questionnaire (taking one to two hours to complete) about symptoms and behaviour recognised in patients with GTS including the following: the presence and types of motor and vocal tics; the age of onset of symptoms; the severity of the tics; suppressibility and duration of the symptoms. The presence of associated behaviour such as coprolalia, copropraxia, echolalia, echopraxia, palilalia, palipraxia, stammering, stuttering, self-injurious impulses, the urge of feeling forced to touch, aggressive behaviour, and sleep disturbances were also inquired after. In addition obsessional thoughts, compulsions and rituals, and other behaviour which may be considered to be obsessional, such as arithmomania and 'evening up', were assessed. Further historical information was obtained by direct interview with reliable informants.

Individuals were assigned to diagnostic categories as follows:

(a) definite GTS: satisfying DSM–III (American Psychiatric Association, 1980) criteria on history and examination
(b) probable GTS: symptoms observed but no history obtained, or history obtained but no symptoms observed
(c) definite CMT: DSM–III diagnosis on history and examination
(d) probable CMT: as for probable GTS but without vocalisations
(e) Obsessive–compulsive behaviour: obtained on history and, in the case of children, corroborated by parents.

Global illness severity was rated according to the following:

(a) mild: present but not requiring medication and not interfering with social life
(b) moderate: present and requiring medication
(c) severe: as for (b) but severely socially disabling. Severely socially disabling in this context means that an individual is unable to live a normal life with respect to employment, interpersonal relationships and living independently.

Statistical analysis

The data were analysed using the χ^2 test and Fisher's exact probability test for contingency tables. The Mann–Whitney *U*-test was used for examining the relationships between

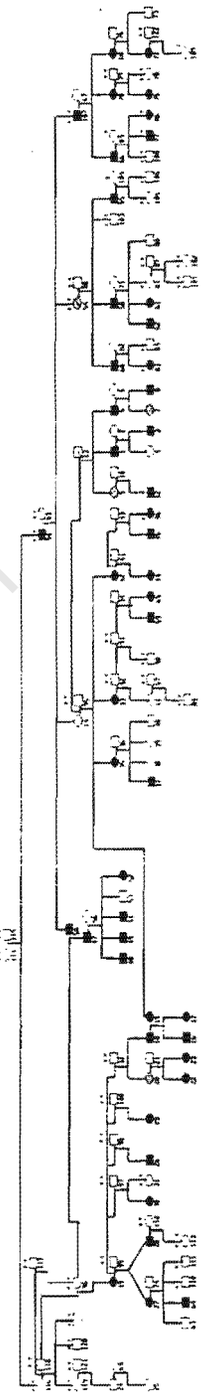

FIG. 1 Pedigree of family with Gilles de la Tourette syndrome (□ man, ○ woman, ● GTS and probable GTS, ◍ CMT and probable CMT, ◍ OCB and probable OCB, X no blood taken, Y not interviewed, Ø deceased).

discrete and continuous variables. Non-parametric tests were employed because the continuous variables were not normally distributed. The data were analysed on the EUCLID computer at University College London, using the MINITAB and EASISTAT statistical packages.

Results

Of 122 members of a family multiply affected by GTS, 85 were interviewed at least once. Fifty-eight were interviewed twice. Six were seen and diagnosed as 'non-cases': five of these were too young for meaningful diagnosis, and one adult, while happy to be seen, was unwilling to answer detailed questions. The family, including members who were not interviewed, are shown in Fig. 1. Information about 31 of these was obtained from other family members, and considered to be sufficient for diagnosis (hence the fact that there are more than the 50 'cases' in the genogram), but not for the analysis of detailed psychopathological data. Information about the remaining six was considered unreliable for the purpose of this communication.

Statistical analysis was therefore performed on 79 individuals about whom there was adequate information. Of the 85 subjects interviewed, 50 were diagnosed as 'cases', as shown in Table I. Cases 27 and 28 were dizygotic twins concordant for GTS. The mean age of the 'cases' was 31.9 years (range 5–84) and that of 'non-cases' was 36 years (range 6–81). Age at onset of symptoms (tics or obsessive–compulsive symptoms) was 8.6 ± 4.6 years (mean + s.d.). For the 50 'cases' (24 men, 26 women), eight had consulted a doctor for an opinion about their symptoms: two for tics; two for a cough; three for behavioural problems, and the index case for GTS at the clinic at the National Hospital for Nervous Diseases, Queen Square. Of these eight, three received treatment from psychiatrists; only one was given drug treatment, following a diagnosis of schizophrenia. Of the 50 'cases' graded for severity, one was graded as severe, one was moderate (index case), and the remaining 48 were mild. For these 50 subjects, associated behaviour was elicited (Table II). Twenty-nine subjects were diagnosed as having definite or probable GTS (Table I). The associated behaviour of these 29 GTS patients is shown in Table III.

The results of the standardised rating scales are presented in Table IV. No significant differences were found in scores between 'cases' and 'non-cases', with the exception of the trait score of the Leyton Obsessional Inventory, which was signficantly higher for the 'cases'. When the scores for the 29 GTS 'cases' only were compared with 'non-cases', no significant differences emerged.

Fifty-two subjects were assessed using the SADS-L, and diagnoses are shown in Table V. Seven subjects received more than one diagnosis. Of the 21 subjects to whom

TABLE I
Diagnoses of affected members

Diagnosis	*No. of individuals*
Definite GTS without OCB	18
Definite GTS with OCB	1
Probable GTS without OCB	9
Probable GTS with OCB	1
Definite CMT without OCB	7
Definite CMT with OCB	2
Probable CMT without OCB	6
Probable CMT with OCB	2
OCB	3
Probable OCB	1
Total	50

GTS, Gilles de la Tourette Syndrome, CMT, chronic motor tic, OCB, obsessive–compulsive behaviour.

TABLE II
Associated behaviours and features of 50 'cases'

Associated behaviour/ feature	*No. of 'cases'*	*No. of 'non-cases'*	*Significance between 'cases' and 'non-cases'*[1]
Sleep disturbance	32	11	NS
'Evening up'	16	0	$P=0.0001$
Forced to touch	11	0	$P=0.0035$
Echolalia	10	0	$P=0.0055$
Aggressive behaviour	10	1	NS
Arithmomania	8	0	$P=0.019$
Echopraxia	8	0	$P=0.0174$
Self-injurious behaviour	6	0	NS
History of stuttering	4	1	NS
Mental coprolalia	3	0	NS
Coprolalia	1	0	NS
Copropraxia	1	0	NS
Palipraxia	1	0	NS
Palilalia	0	0	NS

1. P = Fisher's exact probability test.

TABLE III
Associated behaviour and features of 29 definite or probabl GTS 'cases'

Associated behaviour/ feature	*No. of 'cases'*	*No. of 'non-cases'*	*Significance between 'cases and 'non-cases'*
Sleep disturbance	17	11	NS
'Evening up'	6	0	$P=0.012$
Forced to touch	7	0	$P=0.0065$
Echolalia	7	0	$P=0.0054$
Aggressive behaviour	6	1	NS
Arithmomania	3	0	NS
Echopraxia	7	0	$P=0.0054$
Self-injurious behaviour	5	0	$P=0.038$
History of stuttering	4	1	NS
Mental coprolalia	1	0	NS
Coprolalia	1	0	NS
Copropraxia	1	0	NS
Palipraxia	1	0	NS
Palilalia	0	0	NS

1. P = Fisher's exact probability test.

TABLE IV
Psychopathology assessed by rating scales

Rating scale	*'Cases'*		*'Non-cases'*		*Significance*[1]
	mean	*s.d.*	*mean*	*s.d.*	
Leyton Obsessional Inventory					
Symptom	13.13	8.1	9.86	7.3	NS
	8.90	3.2	7.00	4.1	Trait $U=1216$, $P=0.04$
Total	22.03	10.4	16.86	10.6	NS
Crown Crisp Experiential Index					
Free floating anxiety	5.14	4.2	5.65	4.6	NS
Phobic anxiety	4.62	3.8	4.80	2.7	NS
Somatic anxiety	4.95	3.5	4.25	3.0	NS
Depression	4.41	3.3	4.40	3.7	NS
Hysteria	5.08	3.4	5.15	2.8	NS
Obsessional	5.68	3.2	5.40	2.5	NS
Total	29.86	16.9	29.65	14.3	NS
General Health Questionnaire	4.28	5.9	3.45	5.9	NS
Hostility and Direction of Hostility Questionnaire					
Total	16.61	8.7	14.95	6.4	NS
Direction	−1.03	6.02	−0.84	4.6	NS

1. Mann–Whitney *U*-test.

TABLE V
Psychopathology assessed by SADS–L

Diagnosis	*No. of cases*
Major depressive disorder	10
Alcohol abuse	4
Hypomania episode	3
Generalised anxiety disorder	2
Cyclothymic personality disorder	2
Labile personality disorder	2
Schizophrenia	2
Phobic disorder	1

SADS–L diagnoses were assigned, 15 were 'cases', while six were 'non-cases' ($\chi^2=0.59$; 1 d.f; NS). As there are so few case reports on GTS and schizophrenia coexisting, we briefly present our two cases, though the association may be a chance finding.

Case reports

Case 1

Miss A is 23 years old and single, living with her parents. She was first seen by a psychiatrist in 1986, aged 22, with a presenting complaint of disturbed behaviour, particularly violence. At the time it was noted that she experienced visual and auditory hallucinations, the latter in the second person. Also noted were obsessive–compulsive phenomena, with repeating of the middle letters of words. The initial formulation was that she had a severe personality disorder with "intense ambivalent feelings". A psychotic illness could not be excluded. She was therefore treated initially with oral neuroleptics, then depot long-acting neuroleptics. At interview in late 1987, however, there was evidence of Schneiderian first-rank symptoms, namely auditory hallucinations in the third person, and passivity, with made actions. At that time, when we interviewed her, she admitted to marked obsessive–compulsive phenomena (arithmomania, 'evening-up', intrusive thoughts, and compulsions to move objects), in addition to severe motor and vocal tics (including animal noises), together with coprolalia, copropraxia, echolalia, echopraxia, and palilalia. Her nuclear family corroborated the history of GTS dating back to 12 years of age. This combination of symptoms resulted in severe social disability. She lived at home with her parents, was unemployed, and had no friends.

Case 2

Mrs B, a 58-year-old, separated woman, has had a long history of psychiatric illness since the early 1960s, following the birth of her eighth child. She has warranted at least six in-patient admissions and prolonged attendance at a day hospital, necessitating treatment for many years for schizophrenia with intramuscular flupenthixol decanoate, and oral neuroleptics when required. She currently resides in a community-based psychiatric residential hostel. When interviewed by us for the study she spoke of "Mr Clock", who has spoken to her since 1962, commenting on her actions in the third person. She has also experienced other auditory hallucinations; for example when the traffic is busy she hears a certain man and his associates gossiping about her. These voices are usually intrusive and derogatory. In addition, she experiences thought broadcasting and

passivity. There was also evidence of several negative symptoms of schizophrenia such as blunted affect, avolition, and apathy. On direct questioning Mrs B admitted to the following: finger drumming, looking at her watch constantly without wanting to know the time, and looking in mirrors – all as 'habits'. At interview she cleared her throat several times, and admitted to that and coughing, both as 'habits'. She admitted to arithmomania, in that she had many counting obsessions and had done so since the age of 16. She also admitted to obsessional rituals embracing dressing and undressing, bathing and 'evening-up', in that she felt the need to have symmetry and order. This had begun in her 20s and has continued to date. She was unsure of the age of onset of other GTS symptoms, but they had been present as long as she could remember. It must be noted that she has been on dopamine antagonists for many years for her schizophrenia, which may have masked other motor or vocal tics.

Of note is that three of the bipolar affectives (type II, DSM-III-R; American Psychiatric Association, 1987) are her children: we suggest this is probably as a consequence of assortative mating. Attempts were made to contact the fathers of the children, but letters and telephone calls were consistently unanswered.

Blood samples

Blood samples were collected from 77 family members for DNA extraction. Analysis of linkage with a large series of restriction fragment-length polymorphisms (RFLPs) markers and mini-satellite mapping is in progress. Segregation analysis is also in progress and will be reported in a future communication.

Discussion

Our findings in a large pedigree of many affected individuals, the majority of whom have never sought or received medical attention, would support previous suggestions from both pedigree (Kurlan *et al*, 1987) and epidemiological (Caine *et al*, 1988), data that GTS in the community is more common than previously estimated, usually mild and thus often undiagnosed. Moreover, the frequency of 'caseness' in our pedigree is strikingly similar to that found by Kurlan *et al* (1987), with 54 'cases' in their pedigree of 159 members. The only difference from the Kurlan study is that we included as 'cases' subjects with OCB, but as can be seen from Table I, there were only four such individuals with OCB as a sole diagnosis. We would point out that our number of 'cases' may be an underestimate, as nine who were under the age of 21 were interviewed and labelled 'non-cases', but some of these individuals may well develop GTS at a later age.

Sleep disturbance, which has been documented widely in GTS (Robertson, 1989), was the most common of the associated behaviours, occurring in 32 of our 'cases', but this was not significantly more common than in 'non-cases'. It is noteworthy that 'evening-up' was not only the next most common associated behaviour, but also a significant discriminator between 'cases' and 'non-cases'. Arithmomania, another obsessional characteristic, was also significantly commoner in 'cases' than 'non-cases', as was the feeling or compulsion of being forced to touch. The only other behaviours discriminating between 'cases' and 'non-cases' were echolalia and echopraxia, known to occur in roughly 30–40% of GTS populations (Robertson, 1989) and widely recognised as being integral parts of the syndrome in the initial paper by George Gilles de la Tourette himself in 1885. When the associated behaviours of only GTS and probably GTS 'cases' were compared with 'non-cases', arithmomania failed to reach significance, but self-injurious behaviour was found to discriminate significantly between the two groups. This is particularly noteworthy as we (Robertson *et al*, 1989) have reported self-injury as occurring in one-third of a cohort of 90 GTS patients, suggesting that self-injury in the GTS population has been under-reported to date.

It is important to address the issue as to how typical our 'cases' were in their GTS symptomatology when compared with GTS subjects in other investigations. Our patients exhibited most commonly a variety of OCB, such as 'evening up', arithmomania, and the feeling of being forced to touch, which discriminated between 'cases' and 'non-cases'. This is typical of a substantial proportion of GTS individuals encountered in a wide variety of settings, including clinical (Frankel *et al*, 1986; Robertson *et al*, 1988) and epidemiological (Caine *et al*, 1988) cohorts, as well as pedigree/family studies (Kurlan *et al*, 1986). Thus, our findings suggest our cases are typical and in keeping with the literature, which adds to the argument that OCB is an integral part of the syndrome (Robertson, 1989), irrespective of the severity of GTS symptoms. Sleep disturbance has been reported to be common in GTS subjects, independent of cohort setting, namely epidemiological (Caine *et al*, 1988) or clinical (Robertson *et al*, 1988); it is of importance in this context that it has been argued that GTS is a disorder of arousal (Glaze *et al*, 1982; Barabas *et al*, 1984*a*,*b*, 1985), although there have been opponents to this view (Erenberg, 1985). Other associated behaviours seen in smaller numbers of our pedigree cases were aggression, self-injurious behaviour and, in only one case, copro-phenomena. It would seem that our cases are not unusual in that relatively small numbers of sufferers exhibited these behaviours, and although

such behaviours are documented in patients with GTS, they are more common in those patients who have been referred to physicians and may well be a reflection of the severity of the disorder, and thus a result of ascertainment or referral bias; for the majority of GTS subjects in the community, it is a mild disorder and thus often undiagnosed (Robertson, 1989), and this was the situation with the majority of our cases.

The trait scores of the Leyton Obsessional Inventory were the only ones to discriminate between 'cases' and 'non-cases', thus arguing again for an intimate association between OCB and GTS. Thus, although a significant association between obsessive–compulsive phenomena and core features such as coprophenomena and echo-phenomena has only been documented once (Robertson *et al*, 1988), there is a growing literature on obsessive–compulsive disorder and GTS (Frankel *et al*, 1986; Comings & Comings, 1985; Hagin *et al*, 1982; Montgomery *et al*, 1982; Nee *et al*, 1980; Green & Pitman, 1986).

With regard to the general psychopathology, it can be seen from Table IV that there are no differences in the scores of the rating scales between 'cases' and 'non-cases'. Moreover, the results of the SADS–L show 21 family members had had a psychiatric illness during their lifetime. When doing a breakdown of the specific diagnoses, these are not in excess of the lifetime risks for schizophrenia (1%), major depressive disorder (20%), alcohol abuse (13%), and the others, which could be subsumed under the diagnosis 'neurosis', which has a lifetime risk of 13% (American Psychiatric Association, 1980; Kendell & Zealley, 1983). Moreover, two schizophrenics in a cohort of 122 people is not above the expected (Kendell & Zealley, 1983) and hence our findings support the assertion that there is no connection between schizophrenia and GTS (Robertson, 1989), as has been reflected by only a few case reports in the literature (Caine *et al*, 1978). Our data of depressive disorders, schizophrenia, phobic states, and generalised anxiety not being more common in the GTS population than in the general population does not support the suggestion that these pathologies and GTS are genetically related (Comings *et al*, 1984).

The pattern of inheritance of GTS and CMT in our pedigree supports an autosomal dominant pattern of inheritance, as suggested by previous authors (Devor, 1984; Price *et al*, 1984; Pauls & Leckman, 1986; Kurlan *et al*, 1986, 1987) but this is only evident when the presumed phenotype is broadened to include CMT and OCB. Even when this is done, penetrance is apparently incomplete, as there are two unaffected individuals who, if paternity has been correctly detemined, are obligate carrie of the disease gene (nos 116 and 96). However, pro that GTS, CMT, and OCB are genetically related w have to await the demonstration of linkage betwe GTS and polymorphic genetic markers. The pedigr is a suitable one for linkage analysis because it unlikely that heterogeneity of linkage is presen However, due account for the presence of the tw inbreeding loops (cases 18 married to 15, and al 96 to 50) will have to be taken into account in t calculation of lod scores.

Conclusions

Our pedigree data confirm those of previo investigators suggesting that the majority of GT cases in the community are mild and go undetecte thus the true prevalence of GTS remains unknow Our data also support the notion that GTS ar obsessionality are intimately related. Likewise, ech phenomena are an integral part of the syndrome. W further suggest that self-injurious behaviour occu in a clinically significant proportion of GTS patient that it often goes undetected, and that it can occ in those individuals who have mild GTS symptom Finally, inspection of the pattern of affecte individuals within the family suggests autosom dominant inheritance when the presumed phenoty is broadened to include CMT and OCB.

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Gilles de la Tourette Syndrome in the Middle East
Report of a Cohort and a Multiply Affected Large Pedigree

MARY M. ROBERTSON and M. R. TRIMBLE

In a cohort of five patients from the Middle East with the Gilles de la Tourette syndrome, family history of a tic disorder or the Gilles de la Tourette syndrome was positive in three cases. In one of these there was a multiply affected pedigree spanning six generations. The phenomenology of the syndrome is the same as that described in Western reports. The familial pattern of inheritance and cross-cultural similarity emphasise the biological factors in the aetiology of the syndrome.

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The Gilles de la Tourette syndrome (GTS) is no longer the bizarre rarity that it was once thought to be. Substantial cohorts of GTS patients have been reported from North America, the United Kingdom and Europe, but from most other parts of the world, to date, only case reports exist (Robertson, 1989). The syndrome is diagnosed by the presence of both motor and vocal tics; however, associated features such as echophenomena, coprophenomena, and more recently obsessional thoughts and/or behaviour, are recognised as being integral parts of the syndrome (Lees *et al*, 1984; Robertson *et al*, 1988).

There has only been one report from the Middle East, namely from Saudi Arabia (El-Assra, 1987). This report not only stated that it is the first known documentation from the Middle East, but 'confirmed' supposedly correct previous suggestions that a "disturbed parent–child relationship underlies the psychogenesis of the syndrome", as the "results of the psychotherapeutic sessions and the family meetings . . . showed the patient's inability to grow up psychologically at home, and her use of the GTS symptoms to seek the attention of her parents". Further arguments for the underlying psychogenesis of GTS were advanced by the author who stated that "the early and remarkable response of our patient to haloperidol is doubtful", "because of the small dose of haloperidol administered" (0.5 mg), and "furthermore there was no evidence of organic dysfunction, as indicated by the patient's good health and normal IQ". No mention was made of family history and the only references to cultural factors were that the patient was one of 12 children and that the reason she sought the attention of her parents was because they had always been more attentive to male children – a 'cultural phenomenon'.

While it is acknowledged that sporadic cases of GTS do occur, it is becoming increasingly accepted that in the majority of cases there is a genetic basis to the disorder (Robertson, 1989). In addition, it has been suggested that the anticipated gene for GTS may manifest as GTS or chronic multiple motor or vocal tics only (Pauls *et al*, 1981; Kurlan *et al*, 1986; Robertson & Gourdie, 1990), or obsessional behaviour (Pauls & Leckman, 1986; Pauls *et al*, 1986 *a*, *b*; Comings & Comings, 1987), with obsessional behaviour as opposed to tics being particularly evident in the female relatives of affected families.

A cohort of patients from the Middle East is presented. In addition, a large pedigree with multiply affected members is discussed. We emphasise not only the cross-cultural similarities of GTS patients, in that our patients are not substantially different from those documented in the United States, the United Kingdom or Europe, but note also the similar familial pattern of GTS and/or obsessional disorder in the families of our cohort.

Case reports

Patient no. 1

Miss CM, born in the Sudan, and of Armenian background, presented to the National Hospital, Queen Square, at the age of 22 years. Her first symptom, blinking of the eyes, began at the age of six years. Subsequently, she exhibited shaking of the hands, flexing of various muscles, especially of the hands, nodding of the head, stretching of the neck muscles, shrugging of the shoulders and a complex jumping movement of the whole body. At the age of 15 years she began to have vocalisations such as snorting, coughing and a deep throaty noise. Typically, the symptoms would wax and wane and were made worse by stress and anxiety. She was able to suppress them at the expense of inner tension. She had both echolalia and echopraxia when young (especially if someone made a strange noise or bodily movements), felt forced to touch objects (especially if hot or cold), but exhibited no coprolalia nor obsessive-compulsive behaviour.

The patient was 9 lb (4 kg) at birth and there was no evidence of birth injury. As a child she bit her nails but otherwise her development was unremarkable. Her early life was spent in Africa and she came to England at the age of 10 years. She settled well at school, obtained a degree

equivalent at college and took up a post as assistant to a solicitor with a view to becoming a solicitor. She was single and despite her illness, of which she has always been conscious, she adapted well to life in England. Family history of note is that her father snorted throughout his life and that her brother continually blinked his eyes. On examination, no neurological nor abnormalities of the mental state were detected. She did, however, exhibit several facial and lower limb movements, in addition to the occasional snort. An electroencephalogram (EEG) was normal. She was successfully treated with clonidine (0.2 mg daily), with a marked reduction of all her symptoms resulting in a better quality of life.

Patient no. 2

Miss MA presented to the National Hospital, Queen Square, at the age of 21 years. She was born in the Lebanon. Her symptoms had begun at the age of 10 years with a vocalisation similar to the yelping of a puppy. Since then she exhibited a wide repertoire of motor tics including raising of the eyebrows, blinking, winking, a nasal twitch, sniffing, a movement similar to flicking hair out of her eyes, abdominal contractions, a twisting movement of the torso and leg extension. Subsequent vocalisations have included throat clearing and persistent yelping. She never exhibited copro- nor echophenomena, signs of attention-deficit disorder nor hyperactivity. The only obsessional behaviour she exhibited was the repeated and unnecessary shutting of doors. Typically, the symptoms were increased with anxiety and decreased while playing sport.

Her birth and early childhood were unremarkable. She went to secondary school in Belgium and subsequently attended university in London where she integrated well and achieved well academically. Family history of note is that a paternal aunt was reported to have winked and blinked excessively. Neurological and mental state examinations were normal, apart from facial tics and vocalisations. An EEG and magnetic resonance imaging (MRI) were normal, as were serum copper and caeruloplasmin. As her condition was considered to be mild, no pharmacological treatment was indicated. Reassurance, especially to the family and patient who felt both genetic endowment and the war in Lebanon were responsible for her symptoms, was given, as was supportive psychotherapy.

Patient no. 3

Miss ZA was born in Kuwait of an Arabic background. She presented to the National Hospital, Queen Square, at the age of 22 years. Her first tic was excessive blinking of the eyes at the age of eight years. Since then she has had many motor tics including frowning, raising of the eyebrows, winking, blinking, rolling of the eyes upwards, nasal twitching and flaring, sniffing, several movements of the mouth including pouting, tongue protrusion, facial grimacing, spitting, licking of the lips, head nodding, platysma tightening, shoulder shrugging, pelvic movements, kicking, an abnormal gait interrupted by hopping, complex jumping, jerks of both arms and legs and a complex movement involving hitting of both her head and abdomen. Vocalisations began at the age of nine years and have included yelping, squeaking, coughing, throat clearing and gulping as well as sounds simulating words but which are not recognised as belonging to any particular language. Coprolalia began at the age of 15 years and literal translations of the Arabic words were arse, bitch and pimp. She also had echolalia, echopraxia, palilalia and an urge to touch things. In addition she indulged in self-injurious behaviour such as slapping of her face, punching her head and abdomen and stabbing her hand or face with a pen with resultant bruising. She had exhibited sexual disinhibition in public, exposing her breasts at school and uncovering her thighs in public (this was unacceptable in Moslem culture). She also had obsessional thoughts associated with avoidance behaviour. Her symptoms were worse in public and other stressful situations, and were improved when she concentrated, for example playing the piano. When asleep, the vocalisations disappeared but the tics continued in an irregular fashion. Neurological and mental state examinations were unremarkable. The following investigations were normal: EEG, computerised tomography scan, copper and caeruloplasmin, full blood count and blood chemistry.

The patient's birth and development were unremarkable. Despite interruptions in her schooling because of medical consultations abroad, she was able to attain good grades in high school. She was the ninth of 14 siblings, her mother having had two miscarriages. The family denied any family history of tic or psychiatric disorder. She failed to respond to haloperidol and was successfully treated for a period with metoclopramide although she discontinued this because of an acute dystonic reaction. She was then commenced on sulpiride with some success.

Patient no. 4

Mr MA presented to the National Hospital, Queen Square, at the age of 16 years. He was born in Dubai to Arabic parents. His first symptoms were at the age of 15 years when he had noticed a ticking movement of his right arm. He subsequently had developed frowning, eyebrow raising, rolling of the eyes upwards, a nasal twitch, movements of the upper lip, mouth pouting, a gripping movement of the lips, spitting, licking of the lips, platysma tightening, turning of the head sideways, flicking his hair out of his eyes, a shoulder shrug, extension of the arms, movement of the toes, a whole body jump, looking at his watch as a habit, putting his fingers in his mouth, and although several of these were observed at interview, they were denied by the patient. He admitted to vocalisations including sniffing, gulping and hiccuping, which were evident at the interview. These had developed after the motor tics. He had no coprolalia, copropraxia, self-injurious behaviour, aggression, attention-deficit disorder nor hyperactivity. Of particular interest is that he had many obsessional actions in that he would check a door at least three times even if he knew it was closed, he would tidy his room meticulously to an extent that would worry his family, and he had a bathing ritual in that he had to wash himself in a particular order. Typically, his symptoms were made worse by stress, increased with concentration and since the onset of his

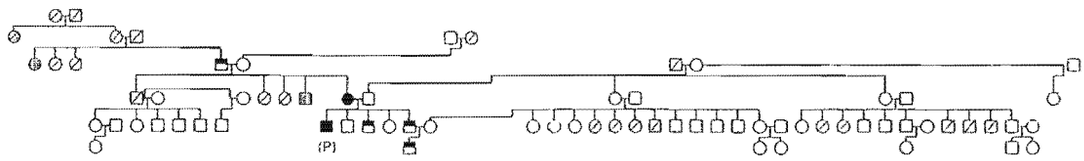

Fig. 1 Arab pedigree multiply affected with the Gilles de la Tourette syndrome chronic tics/habits (▬) and obsessional disorder (▨) (∅ = dead, (P) = (■), the patient).

illness he had only been free from all tics for hours at the most.

He had a normal birth, and apart from walking late, had average milestones. He was brought up by a paternal aunt, but lived only two houses away from the parental home and therefore was in close contact with the family. He was a sensitive child but otherwise no abnormalities were noted. He began school at the age of five years and was still at school at the time of the clinic interview; he got on well with both teachers and peers and apparently had no problems at school. He had several hobbies including playing with model aeroplanes, and he had no trouble making friends.

Family history was obtained by interviewing the patient, his brother and a paternal aunt, and the information obtained revealed a pedigree spanning six generations as shown in Fig. 1. Of note is that his maternal great-aunt was reported to be an obsessive–compulsive hand-washer, as was his maternal aunt. His maternal grandfather blinked his eyes for many years, while his maternal uncle was described as being excessively meticulous as well as a compulsive cleaner. In addition, he was meticulous with regard to the order in his room. The proband's mother, aged 43 years, had motor tics such as jerking of the arms and vocal tics such as clearing of the throat, as well as obsessive–compulsive behaviour in that she would spend two to three hours in the bath; she was a compulsive hand-washer, and if she washed a shirt, she had to do it at least 20 times. He has two brothers who possibly have 'habits'. The unmarried brother had a habit of pinching his body, grinding his teeth, pinching his triceps, pulling the hairs out of his beard, and cutting his nails very deep. His married brother (who was interviewed) admitted to having a 'nervous habit' as a child. His nephew, aged seven years, apparently has started to blink his eyes, stretch his neck, have facial grimaces, look both up and down involuntarily, and also to smell things as a 'habit' (see Fig. 1).

Patient no. 5

Miss NA was born in Iran and presented to the National Hospital, Queen Square, at the age of 12 years. At the age of four years she began to have involuntary movements of the face, head and neck, including excessive eye blinking, which spread to the upper limbs at the age of 11 years. She also had vocalisations including grunting, and her movements would characteristically increase with anxiety. She had had febrile convulsions between the ages of one and five years. She also had a nocturnal convulsion at the age of 11 which was not associated with a fever and was therefore treated with various anticonvulsants including mysoline and tegretol.

The patient was born normally, walked somewhat late, at the age of two, talked at two and was enuretic until aged eight. She was described as a 'nervous child' and had problems at school in that she underachieved and had difficulty in making friends. She was, however, able to read and write. Both her father (aged 40 years) and her mother (aged 30 years), who were unrelated, were well. Her sister (aged 11 years) and her brother (aged four years) were also well. There was no known relevant family history. On examination there were obvious involuntary movements of her face, head and neck, especially excessive blinking. Neurological examination revealed a fine tremor of both hands. There were no Kayser-Fleischer rings. The following special investigations were normal: serology (WR), serum copper, caeruloplasmin and uric acid, skull X-ray and EEG. As she was young and her symptoms were not severe, no medication was given at the time.

Discussion

We present five cases of GTS and a multiply affected pedigree from the Middle East, and suggest therefore that the disorder is probably more common in the Middle East than has so far been recognised – to date there has only been one case documented (El-Assra, 1987). What is perhaps more important, however, is that the phenomenology of the GTS in our cohort is the same as that documented in Western populations, with both motor and vocal tics, obsessive–compulsive behaviour, coprolalia, self-injurious behaviour, echophenomena and the feeling of being forced to touch things (Robertson, 1989). In addition, the familial pattern as documented in case number four is very similar to that encountered in the West (Kurlan *et al*, 1986; Robertson & Gourdie, 1990), and the expression of an anticipated GTS gene seems to have shown itself in our Arab pedigree, presenting as obsessive–compulsive behaviour, GTS or chronic tics, not dissimilar to the pattern found in other large pedigrees (Kurlan *et al*, 1986; Robertson & Gourdie, 1990). This cross-cultural similarity shown by our pedigree when compared with a large variety of world-wide case reports and documentations of large cohorts (for review see Robertson, 1989) emphasises the biological factors in the aetiology of the GTS.

In contrast to cohorts from the West (Robertson *et al*, 1988, 1989), however, none of our patients had associated psychiatric dysfunction (apart from the obsessional symptoms), nor attention-deficit disorder nor hyperactivity. It is also of interest that three of the five cases were female, whereas in most cohorts from the West there is a male to female ratio of 3–4 : 1 (Robertson, 1989). Further studies on patients should resolve these issues.

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Catatonia: Harbinger of the Neuroleptic Malignant Syndrome

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Five consecutive cases of the NMS are presented, in all of which a catatonic state preceded the onset of the condition. Catatonia would appear to represent a highly significant risk factor for the NMS and a possible causal link between the two disorders is suggested.
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The neuroleptic malignant syndrome (NMS) is a well documented complication of neuroleptic treatment. Its major manifestations are muscular rigidity, altered consciousness, hyperthermia and autonomic instability, often accompanied by raised serum creatine phosphokinase (CPK) levels and leucocytosis. Owing to the potential lethality of the NMS, the search for risk factors is urgent. We describe five consecutive patients with the NMS in whom a catatonic state of acute onset appeared to herald the emergence of the syndrome.

Case reports

All five patients were admitted to the psychiatric emergency unit of Groote Schuur Hospital, Cape Town. Table 1 briefly describes the clinical state of each patient before and after the administration of a neuroleptic. None of the patients had received neuroleptics before admission and none had a previous history of psychiatric illness. To exclude another aetiology, the following additional investigations were done – and found to be negative – in every patient after the onset of the NMS: blood culture, toxicology screen, chest X-ray, lumbar puncture, electroencephalography and computerised tomography. In each case the NMS was managed by supportive treatment and without the use of pharmacological

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Normal chromosomal findings in Gilles de la Tourette syndrome

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Sixty-eight consecutive patients (51 male, 17 female) who satisfied DSM-III criteria for Gilles de la Tourette syndrome had blood taken for chromosomal analysis. Three individuals exhibited abnormalities or variations: an XYY chromosome, a heterochromatin of chromosome 1, and heterochromatin of chromosome 9, respectively. The remaining 65 patients showed apparently normal karyotypes without any striking heteromorphisms. The literature on chromosomal abnormalities in Gilles de la Tourette syndrome and associated disorders is reviewed.

Keywords: Chromosomes – Genetics – Gilles de la Tourette – Obsessive compulsive disorder – Tics

INTRODUCTION

Prompted by the report of a patient with Gilles de la Tourette syndrome (GTS) from the National Hospital for Nervous Diseases, Queen Square, London (NHND), who also had an XYY chromosomal (Merskey, 1974), we undertook a prospective investigation, performing chromosomal studies on 68 consecutive patients with GTS who satisfied DSM-III (American Psychiatric Association, 1980) criteria for GTS.

Three individuals exhibited karyotype deviations: (1) a sex chromosome anomaly (an extra Y chromosome in a male); (2) a heteromorphism of chromosome 1 (1qh+); and (3) a heteromorphism of chromosome 9 (inv 9) (p11q13). The remaining 65 patients showed apparently normal karyotypes without major heteromorphisms.

The diagnostic chromosomal studies were carried out on G-banded chromosomes (method of Seabright, 1971) karyotyped according to International Nomenclature (ISCN, 1981) and performed by Dr D.T. Hughes.

The three patients described here were noteworthy in that they had striking heterochromatic chromosomal features through duplication of a band (1qh+), structural rearrangement (inv 9qh) or an extra chromosome. C-banding (method of Arrighi and Hsu, 1971) confirmed the expected constitutive heterochromatic nature of these G-banded polymorphic variants.

The significance of these findings is discussed following the brief presentation of the clinical features of these patients.

CASE REPORTS

Patient 1

Mr T attended NHND at the age of 15. He was born normally weighing 7 lb. and although his motor development was normal, by two and a half he was only saying a few words. He was hyperactive as a child, often screaming and prone to rage attacks. He was phobic of the dark, and has always bitten his nails. Since early childhood, his parents noted that he was "always twitching", with the movements affecting his face; these included mouth opening, head extension and a moderate range of simple movements affecting his shoulder, trunk, proximal arm and legs. He also exhibited sounds such as throat clearing, sniffing, snorting and coprolalia (the involuntary inappropriate uttering of obscenities). At the age of 11 he developed obsessional behaviour, for example unnecessarily folding his clothes neatly into piles, putting records in files and, during interview, he tidied the examination room, and folded his clothes before he could lie down on the couch.

He attended a normal school until the age of 7, but made little progress, and was therefore sent to a special school, where he coped for 8 years. He then over a year became uncontrollable with violent outbursts of behaviour, usually triggered by provocation. He became destructive to property and was only rarely violent to people. There was no relevant known family history.

Examination of his mental state was normal. He exhibited multiple motor and vocal tics. Psychometric testing revealed that his verbal IQ was 52 and performance IQ 48:

his Schonell word reading test was managed at the 7.7 year level and Schonell spelling was commensurate with this. A CT scan and both chest and skull X-rays were normal. An EEG was abnormal with excess of slow activity. Routine haematological investigations were normal. Chromosomal investigations revealed a 47,XYY karyotype.

Patient 2

Miss S presented to the NHND at the age of 17. Her GTS symptoms began at the age of 8 with throat clearing. Since then her repertoire of motor tics has included eye blinking, eyes rolling upwards, twisting of the upper lip, head nodding, a pulling in of the abdominal muscles, and foot tapping. She has several vocalizations including a loud hooting noise, snorts, sniffs and throat clearing. Her symptoms characteristically wax and wane in severity, are worsened by anxiety and improved by concentration, and she is able to suppress them voluntarily at the expense of inner tension. She feels the urge to touch things, but has never exhibited copro- nor echophenomena.

She denied any relevant family history of tics, psychiatric or neurological disorder. She was born normally, weighing 7 lb. 14 oz after a normal pregnancy, but labour was induced. She had average milestones and coped with life until she was 10 years old, when it became necessary for her to attend a special school: she nevertheless obtained two CSEs, leaving school at 16 years, after which she attended a college for further education.

Neurological and mental status examinations were normal. Neuropsychological testing showed a verbal IQ of 79 and a performance IQ of 87. CT scan was normal. An EEG was abnormal, with brief episodes of generalized slow activity with components at 3 cycles/s as well as theta activity, which lasted up to 2 s, although during overbreathing more protracted episodes occurred when, at times, the slow waves were associated with sharp components. Full blood count, urea and electrolytes, liver function tests, copper and caeruloplasmin were all within normal limits. Chromosomal analysis revealed 46,XX 1qh+ female karyotype (G-banded) with a heteromorphic chromosome 1, having extra heterochromatin next to the centromere in the long arm, which was considered to be a familial polymorphism.

Patient 3

Miss W presented to the NHND at the age of 20. Since the age of 8, she displayed GTS symptoms, beginning with excessive blinking of the eyes and also exhibiting head nodding, rotation and tilting back, shoulder shrugging, movements of the upper and lower limbs, sniffing and coughing. She had no history of copro- nor echophenomena. Family history information was unremarkable. She was born normally weighing 5 lb. 4 oz. As a teenager she was diagnosed as having anorexia nervosa. She subsequently described depressive mood swings and panic attacks. Apart from multiple tics and vocalizations, examination of her mental state was normal, as was her neurological examination. Neuropsychological assessment gave a verbal IQ of 103 and performance IQ of 100. A CT scan and EEG were normal. All routine blood tests including serum copper and caeruloplasmin were within normal limits. Chromosomal analysis revealed 46,XX,inv(9)(p11;q13) normal female karyotype, with a heteromorphic chromosome 9 which had a pericentric inversion involving transfer of the heterochromatic long arm band (9qh) to the short arm (p) (G-banded): this feature was considered likely to be a familial polymorphism.

DISCUSSION

Gilles de la Tourette syndrome (GTS) is a disorder consisting of multiple motor and one or more vocal (phonic) tics (American Psychiatric Association, 1987) and is no longer considered to be rare (Robertson, 1989). GTS is currently thought to be genetic and inherited as an autosomal dominant gene, with variable expression and sex-specific penetrance (Comings *et al.*, 1984; Devor, 1984; Price *et al.*, 1984; Pauls and Leckman, 1986; Pauls *et al.*, 1990; Curtis *et al.*, 1992). Pauls and Leckman (1986), moreover, pointed out that the phenotypic spectrum of GTS subdivided into three distinct diagnostic groups: GTS, chronic multiple tic syndrome (CMT) and obsessive compulsive disorder (OCD), all being regarded as alternative phenotypic expressions of the putative GTS gene. Other family, clinical and epidemiological studies also suggest that GTS and OCD or obsessional compulsive behaviours (OCB) are related (Montgomery *et al.*, 1982; Pauls *et al.*, 1986a, b; Kurlan *et al.*, 1986; Caine *et al.*, 1988; Robertson *et al.*, 1988; Robertson and Gourdie, 1990).

A range of chromosomal anomalies in GTS has been reported. Thus Merskey (1974) described a man with an XYY karyotype pattern. Singh *et al.* (1982) reported a Black woman with GTS with triple X and 9p mosaicism. Robertson *et al.* (1989) documented a patient with GTS and a 3/8 balanced translocation, but on examination of family members, GTS was not found to co-segregate with the chromosomal abnormality (Brett *et al.*, in preparation).

Taylor (1990) reported the case of a 16-year-old male with GTS and a 9p terminal deletion (9)(qter p2304). The patient had negative family history (father 46,XY and mother 46,XX), was born 3 weeks prematurely, a breech presentation by Caesarean section. He was developmentally retarded and had the dysmorphic features of the 9p monosomy deletion syndrome, in addition to many classic features diagnostic of GTS. Kerbeshian *et al.* (1984) described two patients with GTS with the fragile X-syndrome. Six patients with GTS and Down's syndrome (trisomy 21) have also been reported (Sacks, 1982; Barabas

et al., 1986; Karlinsky *et al.*, 1986 Collacott and Ismail, 1988).

Comings *et al.* (1986) reported a family in which six members with "varying manifestations" of GTS all had a 7q22:18q22.1 balanced translocation. Bearing in mind the recent suggestions that obsessional illness and GTS are closely related, stemming from psychopathological, family and pedigree studies (see above), it is of added interest, therefore, that cytogenetical analysis of a woman presenting with OCB, some hyperactivity, panic attacks and visual hallucinations, in addition to other behavioural problems revealed a deletion of the long arm of chromosome 18 at 18q22.2 breakpoint (Donnai, 1987).

There are several explanations for the occurrence of the chromosomal abnormality (XYY) and variations in our GTS patients, and indeed those in the literature.

As GTS is quite common, the possibility must exist that the chromosomal abnormalities found could have occurred by chance alone. The polymorphisms may also be regarded as variations of normal. As familial polymorphisms do occur, the variations in the second and third patients in our study could very easily have been due to this, and indeed this was suggested in the cytogenetic reports. A limitation of the present study is that no detailed family investigations (interviews or blood samples) were performed. However, as in the vast majority of patients in clinical studies there is a positive family history (Robertson *et al.*, 1988; Robertson and Gourdie, 1990), the absence of a positive family in these patients (and many in the literature on GTS and chromosomal abnormalities) is support that the chromosomal anomalies may be relevant.

None of the patients who had chromosomal abnormalities in our study were absolutely typical of GTS. The third patient had a history of anorexia nervosa, mood swings and panic attacks. Despite classical GTS manifestations, the first patient in our study is, however, severely learning disabled (mentally retarded) and the second patient is moderately disabled. In these two latter patients, the chromosomal abnormality and variation could have been related to this disability rather than to the GTS. Patients in other reports of chromosomal abnormalities and GTS have been learning disabled, supporting the argument that the learning disability and the chromosomal abnormalities may be related. Although GTS has been reported in learning disabled patients (Golden and Greenhill, 1981; Reid, 1984) it is uncommon: this is possibly due to underdiagnosis, as learning disability has been reported in 10% of one GTS cohort (Golden and Hood, 1982).

It has been postulated by Comings *et al.* (1986) that GTS is due to a deletion of chromosome 18. However, in the present study of 68 GTS patients we did not find an 18q- deletion in standard G-banded chromosome preparations, analysed in the banding range of 368 to 529 bands per haploid karyotype of the International Nomenclature as used in routine cytogenetical diagnosis (ISCN, 1981). Such a large deletion as the 18q22.2 to 18qter should not have been overlooked in our standard G-banding at the minimal level of 368 bands per haploid karyotype. This supports the data of Heutink *et al.* (1990) who found no evidence for genetic linkage on chromosome 18 and chromosome 7 in six extended families. Data from the markers tested made it possible to exclude the whole of chromosome 18 and the chromosome 7q21.3-qter region as a site for the GTS gene (Heutink *et al.*, 1990). However, it is possible that smaller deletions of 18q could be detected by further studies employing higher resolution banding. Furthermore, application of techniques of molecular genetics employing gene probes could detect submicroscopic microdeletions and also microduplications such as those recently reported in three patients with Down's syndrome lacking apparent trisomy (Delabar *et al.*, 1987).

All three of the GTS patients in our consecutive series presented here have a chromosomal anomaly involving a deviant amount or location of heterochromatin. This is any chromatin characterized by an excessive amount of desoxyribose-type nucleoprotein, with a looser structure, and functionally more generalized than the more specific action of genes in the euchromatin which gives the familiar Mendelian ratio. Thus, patient number one (47,XYY) in having an extra Y thereby has a striking increase in heterochromatin because a large part of the long arm of the Y is normally heterochromatic. Both our patients with a variant chromosome have either an extra amount of heterochromatin, as in the 1qh+ (Patient 2), or a rearrangement of heterochromatin, as in the pericentric inversion of chromosome 9 (Patient 3). Although we were unable to carry out family studies, it is believed that these features are familial polymorphisms because variants of this kind are known to occur in other families (Hook and Porter, 1977). Although these polymorphisms could occur by chance, it is sufficiently noteworthy in our sample (i.e. 3%) to warrant further study because they have previously been reported to occur at lower frequencies in population studies, for example in White Europeans 1qh+ = 0.09%, inv9 = 0.73% (Hsu *et al.*, 1987).

CONCLUSIONS

In conclusion, although no one chromosomal abnormality can be said to be characteristic of GTS, it is noteworthy that an XYY abnormality has been documented twice; chromosome 9 has been reported as abnormal three times; chromosome 18 has been implicated in six cases in one family in one centre, and in a patient with obsessional disorder in another centre. An abnormality of chromosomes 3 and 8 has been documented once. Very many of these chromosomal abnormalities and variations could be chance association, except where there is co-segregation of the cytogenetic abnormality and GTS in the family.

The present findings raise questions about the role of constitutive heterochromatin and familial polymorphisms, which may well merit further attention in GTS as possible predisposing factors affecting the expression of genes causing GTS.

Finally, as more than 50% of the genome has now been excluded (Pakstis *et al.*, 1991), the definition of the GTS phenotype must also be addressed in future investigations. From the point of the clinician, when a GTS patient is not typical, and specifically when the individual is learning disabled, chromosomal analysis is suggested, as this may shed further light on the genetics of GTS.

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Autosomal Dominant Gene Transmission in a Large Kindred with Gilles de la Tourette Syndrome

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A multiplex kindred ascertained through a single proband with GTS has been systematically investigated with standardised diagnostic instruments for other cases of GTS and related disorders. Complex segregation analysis supported the hypothesis that a single major gene inherited in autosomal dominant fashion but with incomplete penetrance contributed most of the variance in the liability to develop GTS and related disorders. This result is consistent with previous segregation analyses which have employed different methods of ascertainment, and tends to confirm that a proportion of GTS is due to a dominant gene and is suitable for investigation with genetic markers for linkage analysis.

The first clear medical description of the Gilles de la Tourette syndrome (GTS) was made by Itard in 1825. In 1885 Georges Gilles de la Tourette described nine cases of the syndrome. He emphasised the triad of multiple tics, coprolalia and echolalia, noted the association with obsessive-compulsive behaviour (OCB), and considered the condition to have a hereditary component.

There have been many investigations of genetic predisposition to GTS, but such studies did not, until recently, employ modern methods of segregation analysis. Some researchers have commented that GTS is often sporadic (Baraitser, 1982; Zausmer & Dewey, 1987) suggesting incomplete penetrance, the existence of non-genetic phenocopies, or cases caused by new mutations. Evidence for a genetic factor can be inferred from twin data if it can be assumed that the twin method is valid for studying GTS. Robertson (1989) has reported 11 concordant monozygotic (MZ) twin pairs, and Price *et al* (1985) reported a concordance of 0.533 for MZ twins but only 0.077 for dizygotic twins. In addition, numerous family studies indicate that the relatives of probands with GTS may present with either GTS or motor or vocal tics only, and some studies provide evidence that within these families chronic multiple tics (CMT) and GTS are related genetically (Robertson, 1989). Two large pedigrees have been reported previously. In the first, 17 out of 43 members were affected (Guggenheim, 1979). In the second (Kurlan *et al*, 1986, 1987; Pauls *et al*, 1990), 159 family members were evaluated and 54 subjects were diagnosed as definite or probable GTS or CMT.

The mode of transmission for GTS and CMT can be investigated using segregation analysis on the relatives of affected probands. Four such studies (Comings *et al*, 1984; Devor, 1984; Price *et al*, 1984; Pauls & Leckman, 1986), on independent samples, have suggested that a dominant autosomal gene contributes to liability. Comings *et al* (1984) found penetrance to be low but Pauls & Leckman (1986) found it to be high. The discrepancy may have arisen because Pauls & Leckman used fairly stringent criteria, whereas Comings *et al* included many other disorders such as agoraphobia, panic attacks, mania, depression and schizoid behaviour within the GTS spectrum (Robertson, 1989). Pauls *et al* (1990) carried out a complex segregation analysis on one large multiply affected pedigree, and concluded that cases of GTS and CMT within the pedigree were likely to be due to an autosomal dominant gene with penetrance similar to that reported by Pauls & Leckman (1986). The estimates for penetrance of GTS were 0.81 for males and 0.31 for females, whereas if cases of CMT were also included as affected the penetrance estimates rose to 0.99 and 0.60 respectively.

We have identified, through the index case who presented at the Neuropsychiatric Clinic at the National Hospital for Nervous Diseases, Queen Square, a British pedigree of six generations and 122 members. We report our findings from segregation analysis, testing several genetic hypotheses for the transmission of GTS including single gene transmission, multifactorial transmission, and the mixed model.

Method

The index patient with GTS presented with his mother, who told the doctor that she knew of a few relatives with similar symptoms. These were interviewed, and it became clear that the family would be of interest for the study of the genetics of the illness, and in particular for linkage analysis. The pedigree was originally selected for study on the basis that it contained multiple cases, and as such could not be

considered ideal for segregation analysis. Nevertheless, subsequently subjects were included regardless of their affection status, and there did seem to be a uniformly high density of cases throughout the pedigree rather than only in those branches which had led to its selection.

As many as possible of the pedigree members were interviewed personally. Those who were not available for interview but about whom information could be gained from relatives were assigned a 'best guess' diagnosis.

In accordance with the procedure described by Morton *et al* (1983), the pedigree was divided up into nuclear families before analysis. Each nuclear family which did not have the original proband as a member was assigned a 'pointer', this being defined as the affected person who had led to that family being included in the analysis. Because of the failure to use a systematic method for extending the pedigree, the concept of a pointer was notional only. The proband was taken to be the pointer for the family of the most closely related affected case, and then affected members of the latter's family were used as pointers to other families, and so on until all cases had been included. Nuclear families were included in the analysis if there was an affected child or parent, but not if no members were affected.

Once the pedigree was divided into nuclear families, the data were analysed according to the mixed model of Morton & MacLean (1974) as modified in the computer program POINTER (Lalouel & Morton, 1981; Morton *et al*, 1983). The mixed model proposes that affection results from the additive contribution of three factors: a random environmental component, multifactorial transmission (not necessarily genetic), and a single major locus effect. Each factor may or may not contribute to the liability to a particular disease. Multifactorial transmission may be due to a large number of independent factors, which may be polygenic or cultural, or both, and results in a general tendency for the child to resemble the parent. Single major locus transmission occurs as a result of the inheritance of alleles at one particular locus. It is assumed that there are two alleles, a normal allele and a disease allele, and that the three possible genotypes at this locus (AA, Aa or aa) lead to significantly different liabilities to develop the disease.

Single major locus transmission thus depends on the discrete phenomenon of whether or not a particular allele is inherited from the parent, and as such it behaves somewhat differently from multifactorial transmission. For example, there is a tendency for single-locus effects to be 'better preserved' than multifactorial effects as an extended pedigree is traversed – if a rare disease is due to multifactorial transmission then the risk to the relatives of secondary cases (those ascertained through a proband) is less than the risk to relatives of the proband, but if the disease is due to a single susceptibility allele then the risk to the relatives of all cases is the same.

POINTER allows constraints to be applied to the mixed model so that the likelihoods of different modes of transmission can be compared. If this is done with extended pedigrees (such as the one reported here) it is possible to test whether there is evidence for familial transmission, whether there is evidence for a multifactorial component, and whether there is evidence for a single major locus component.

Diagnosis

One of us (MMR) evaluated the pedigree over two year Subjects were interviewed to assess 'caseness' using semistructured interview (Robertson & Gourdie, 1990) an were assigned to diagnostic categories as follows:

(a) definite GTS – satisfying DSM-III (America Psychiatric Association, 1980) criteria on history an examination
(b) probable GTS – symptoms observed but no histo obtained, or history obtained but no sympton observed
(c) definite CMT – DSM-III diagnosis on history an examination
(d) probable CMT – as for probable GTS but witho vocalisations
(e) obsessive-compulsive behaviour – obtained on histo and, in the case of children, corroborated b parents.

Method of analysis

The pedigree was inspected and affection rates and s distribution of the different syndromes were studied. O of us (DC) used POINTER to investigate different models aetiology. Each analysis was performed taking either GT or probable GTS as indicating affection, then was repeate taking GTS, probable GTS, CMT, and probable CMT indicating affection. The analyses were repeated using wide range of different population prevalences and of s ratios (male prevalence for GTS ranging from 10% 0.05% and male:female ratio ranging from 1:1 to 5:1). C each occasion the prevalence for children of age seven under was taken to be half the corresponding adu prevalence. To compare competing pairs of hypothese iteration was performed for each hypothesis and the l of the ratio of the maximum-likelihood estimates of t two hypotheses was obtained. This was taken to appro mate to a χ^2 statistic with the number of degrees freedom equal to the number of constraints remov between one hypothesis and the next. The following pai of hypotheses were compared:

(a) no parent–child transmission against multifactori transmission
(b) multifactorial transmission against the mixed mod (including a major locus effect and multifactori factors)
(c) single gene (major locus) transmission against t mixed model
(d) single gene recessive transmission against single ge dominant transmission.

The following pairs of hypotheses were compared usi realistic values for population prevalence and sex ratio, a using the maximum-likelihood estimates of other par meters derived from the earlier analyses:

(a) different gene frequencies producing either GT alone on the one hand, or GTS and CMT on t other, against equal gene frequencies (i.e. possib the same gene producing both illnesses)

(b) Mendelian transmission probabilities against non-Mendelian probabilities.

The results of the former of these two analyses were also used to derive maximum-likelihood estimates of gene penetrance. The second of the above analyses was performed as a test of goodness of fit.

Results

The pedigree comprised 122 people, of whom 85 were interviewed, and information about a further 22 members was obtained and was considered to be sufficient for diagnosis. Information about the remaining 15 was considered unreliable. Clinical and psychopathological features of the pedigree, including a genogram, have been presented previously (Robertson & Gourdie, 1990).

Table 1 shows that the male:female ratios for both GTS and CMT in our sample were approximately equal, with only a slight male excess. Using a modified χ^2 test it was possible to assign a 95% upper confidence limit for the true male:female ratio for the prevalence of GTS of slightly less than 2:1 (that it could be as high as 3:1 was rejected at $P<0.01$).

Table 1 shows that there was a slight, non-significant excess of women with obsessive-compulsive symptoms. All five subjects who had a diagnosis of OCB or probable OCB without GTS or CMT were female. Since this is in line with previous work suggesting that OCB alone is more common in female than male relatives of patients with GTS, this finding has a one-tailed significance of $P=0.031$.

Inspection of the pedigree suggested a dominant gene with partial penetrance, although in fact there are more affected individuals in the pedigree than would be expected under these conditions. Using POINTER for formal segregation analysis, the hypothesis that there was no transmission of a liability to affection from parent to child was rejected at $P<0.001$ for both GTS and CMT at all sex ratios and all population frequencies.

Table 1
Numbers of cases of Gilles de la Tourette syndrome (GTS)[1], definite and probable chronic multiple tics (CMT)[2], and definite and probable obsessive-compulsive behaviour (OCB)[3], by sex

	Male	Female	Total
GTS	16 (15%)	13 (12%)	29 (27%)
No GTS	44 (41%)	34 (32%)	78 (73%)
GTS + CMT	26 (24%)	23 (22%)	49 (46%)
Neither	34 (32%)	24 (22%)	58 (54%)
OCB	10 (9%)	13 (12%)	23 (21%)
No OCB	50 (47%)	34 (32%)	84 (79%)
	60 (56%)	47 (44%)	107

1. $\chi^2=0.030$, d.f. = 1, NS.
2. $\chi^2=0.407$, d.f. = 1, NS.
3. $\chi^2=1.973$, d.f. = 1, NS.

The hypothesis that there is no single major locus could be rejected at $P<0.01$ for all sex ratios if the true prevalence of GTS among males is 1% or less. This hypothesis could be rejected at $P<0.001$ if the true male prevalence of GTS is 0.5% or less, or if the male prevalence of CMT is 5% or less. In other words, there is good evidence in favour of transmission by a single major locus, and this evidence is not particularly dependent on the population prevalence nor the male:female ratio specified.

When the model was unconstrained (i.e. the mixed model of transmission was specified) the maximum-likelihood parameters obtained favoured a single-locus mode of transmission, without any additional multifactorial transmission. In addition, for all male prevalence values for GTS of less than 5%, a fully dominant mode of transmission was preferred, and when fully dominant transmission was compared with fully recessive transmission, the latter could be rejected at $P<0.001$.

With the exception of unrealistically high prevalences, the evidence for major locus dominant transmission was not contingent on the population prevalence or the male:female ratio specified. However, the same cannot be said about the estimates obtained for the frequency of the disease allele, which were highly dependent on these parameters. In fact, once the disease prevalence is set to be 1% or less, the maximum-likelihood estimate for the allele frequency is directly proportional to the male prevalence supplied. The value obtained for the constant linking the two is set out in Table 2 for the different male:female ratios and different disease models used.

The data were analysed in more detail using what we believe to be close to the true values for population prevalence: a prevalence among adult males and females of 0.0005 for GTS, and twice that for the combined syndromes of GTS or CMT. This yielded maximum-likelihood estimates for the disease allele frequency of 0.0004 if GTS only was studied, or 0.0006 if GTS and CMT cases were included. Since logically if both diseases represent different expressions of the same disease allele, the allele frequencies must be equal in each case, the allele frequency was constrained to be 0.0005 and the analysis was repeated. The likelihoods of the models did not change appreciably, and so the data could be said to be consistent with the hypothesis that the different diseases represent variant expressions of a single dominant gene. The penetrance of this gene was estimated to be 0.50 if GTS is taken to indicate affection, or 0.88 if either GTS or CMT is indicative of affection. The estimates for the probability of affection in the absence of the disease allele (the normal homozygote

Table 2
Value of constant, c, relating estimated gene frequency, q, to male population prevalence specified, Kp (for all male prevalences of 1% or less) ($q=cKp$)

Sex ratio (male:female)	GTS	GTS + CMT
1:1	0.85	0.58
2:1	0.63	0.39
5:1	0.52	0.14

penetrance) and for the probability of an affected individual not having the disease allele (the proportion of phenocopies) were both very low (less than 0.001). However, these last two results do not refer to the population in general but only to individuals within the pedigree, and so little reliance should be placed upon them when compared with the results of studies that have taken a wider sample of cases.

Finally, the constraints on transmission probabilities were removed to allow for non-Mendelian transmission. The models using the realistic values for prevalence as described above were tested under these conditions. In each case the unconstrained model was significantly more likely than the model constrained to Mendelian transmission, suggesting that the mixed model did not fit well with our data.

Discussion

Previous workers have suggested that GTS is a genetically heterogeneous disease, with some cases being clearly familial and apparently having a significant single-locus effect, but with a substantial fraction of cases being sporadic and of unknown aetiology. Since GTS is rare it seems reasonable to regard the pedigree presented here as being representative of a single genetic subtype, and therefore offering a particularly valuable opportunity to study a genetically homogeneous form of the disease.

There are strong theoretical objections to performing genetic studies, and particularly segregation analysis, on a pedigree which has been selected on the basis of containing multiple affected cases, since this introduces an obvious ascertainment bias. The likely effects of such a bias are impossible to quantify because the pedigree was not sampled or extended in a systematic manner, and so it is not possible to be certain of the extent to which some of our results may be artefactual. However, we believe that there are benefits in being able to study the characteristics of a genetically 'pure' form of the disease such as this pedigree seems to represent, and that these benefits outweigh the disadvantages of having to use a method which is not strictly appropriate. It is perhaps also important to realise that if there is genetic heterogeneity then the application of segregation analysis to a series of systematically ascertained probands would also be inappropriate. In such a case the parameters obtained would represent a compromise between subtypes rather than representing a true picture of the mode of transmission in any one family. In the present case we do not think it is feasible that the results we have obtained could be solely due to the methodological weaknesses outlined.

The results of our analysis are similar to those obtained by other workers who have analysed a systematic series of families (Pauls & Leckman, 1986 and one large pedigree (Pauls *et al*, 1990). The onl slight anomaly in our data was that we did not detec the excess of male cases of GTS that is commonl described. This may represent a genuine differenc between the form of the disease in this pedigree an other forms in the population, or may be due t chance or some other cause. The subsequen specification of equal prevalences for both sexe meant that the penetrances we derived for males an females were also equal.

There was strong evidence for the action of a majo autosomal dominant locus, and this was not sensitiv to the disease prevalence specified. It is not clear wh this model was shown to fit poorly when the constraint on Mendelian transmission were removed, althoug this may possibly be due to the rather high frequenc of cases commented on earlier. In general our result provide further support for the theory that some case of GTS are due to an autosomal dominant gene tha can also be expressed as CMT, which seems, there fore, to be a milder form of the same illness.

Because there were only five cases of OCB in th absence of GTS or CMT, it was not possible formall to investigate a genetic relationship between th syndromes. Nevertheless, the occurrence of five case in a sample of this size represents a significan increase over the normal prevalence. It is noteworth that under the assumption of autosomal dominan transmission four of these five cases were 'obligat carriers' for CMT and GTS – that is, people wh while not having tics themselves had affected parent and siblings, and appeared to pass the syndrome t their children. These observations add weight to th suggestion that OCB alone can occur as a varian expression of the genetic abnormality which gives ris to GTS and CMT. There is also some evidence fo the hypothesis that such expression occurs mor frequently in females than males, as all five suc cases in our sample were female.

It is not possible to determine the extent to whic the results from the study of one multiply affecte pedigree can be generalised. Nevertheless, there wer no obvious phenomenological differences betwee these cases and those described in the literature o encountered by MMR in other circumstances, an the genetic parameters derived from this pedigre conformed reassuringly closely to the results o similar segregation analyses which have employe different ascertainment procedures. It may, therefore be the case that a substantial majority of GTS is du to the same genetic mechanism as that which i operating in this pedigree.

The indication that a single-gene predisposition t GTS is operative in this large pedigree means tha

it is feasible to find genetic markers linked to GTS. The parameters that have been estimated in the segregation analysis can be employed in the models to be used for linkage analysis. If markers linked to GTS can be found, this may lead to the cloning and sequencing of the responsible mutation. New preventive strategies and more effective treatments for this distressing disorder might then become possible (Gurling, 1985, 1986).

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Evidence for Autosomal Dominant Transmission in Tourette's Syndrome
United Kingdom Cohort Study

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Complex segregation analyses were performed on families ascertained through 40 unselected consecutive patients with Tourette's syndrome to examine the hypothesis that its transmission is consistent with genetic inheritance. Analyses were done using several diagnostic classifications. All results were consistent with an autosomal dominant gene with high penetrance. The penetrances ranged from 0.882 to 1.000 for males and 0.452 to 0.980 for females, depending upon the specific classification scheme incorporated into the analyses.

Tourette's syndrome (TS) is characterised by multiple motor and one or more vocal tics of more than a year's duration and an age of onset before 21 years (American Psychiatric Association, 1987). Early family studies suggested that a single major gene confers susceptibility for TS (Baron *et al*, 1981; Kidd & Pauls, 1982; Comings *et al*, 1984; Devor *et al*, 1984; Price *et al*, 1984; Curtis *et al*, 1992). However, findings from these studies were inconsistent as to the precise mode of inheritance. Some suggested autosomal dominant inheritance with reduced penetrance, others were consistent with polygenic inheritance, while others suggested that the genetic model was additive. Pauls & Leckman (1986) performed complex segregation analysis on a sample of 27 families with TS sufferers in which all available relatives were personally interviewed. These investigators reported that an autosomal dominant model best described the transmission. Subsequent analyses of large kindreds with TS (Pauls *et al*, 1990; Curtis *et al*, 1992) also supported the hypothesis of a major dominant gene with high penetrance.

However, studies of large kindreds need to be interpreted with the knowledge that the bias introduced by the ascertainment of such multiplex families cannot be easily incorporated into the analyses. A much better test of genetic transmission can be accomplished with a larger sample of smaller families that have been ascertained without regard to familial loading. The present study was undertaken to examine the inheritance pattern of TS in a UK cohort ascertained through consecutive admissions to a TS clinic.

Method

Families included in this study were ascertained through 40 consecutive new cases of TS registered at the National Hospital for Neurology and Neurosurgery (NHNN), Queen Square, London, over eight months. Two additional patients were seen in this time, but their families were not included in this study as information was not available on the biological relatives. In all 40 families, direct clinical interviews were conducted with the index case (by MMR) and all living relatives (VE). Data were obtained about all first-degree relatives from each proband (and parents/other relatives) during the initial interview at the clinic. These data were included in the final diagnostic estimates for each relative. All subjects were interviewed to assess 'caseness' using a semistructured interview by one of the authors (VE) who is well acquainted with the interview schedule widely used (Robertson *et al*, 1988; Robertson & Gourdie, 1990).

Complex segregation analyses were completed using the unified model as implemented in the computer program POINTER (Lalouel *et al*, 1983). The unit of analysis in POINTER is the nuclear family. Thus, multigenerational families were broken down into smaller units. The 40 ascertained families comprised 49 nuclear families. Pointers (i.e. affected probands) to nuclear families indicate how each family is related to the proband's nuclear family (Lalouel & Morton, 1981). The unified model as incorporated in POINTER has five main parameters: q, the frequency of the putative major gene; d, the degree of dominance of the putative major gene; h^2, the heritability of the polygenic component important for the expression of the disorder; t, the major-gene effect measured as the distance between two homozygotes; and *tau*, the probability that the risk allele will be transmitted by the heterozygous genotype.

Segregation analyses were carried out for five different diagnostic schemes:

(a) TS only
(b) TS or chronic multiple tics (CMT)
(c) TS, CMT or transient tic disorder (TTD)
(d) TS or obsessive–compulsive behaviour (OCB)
(e) TS, CMT, TTD or OCB.

OCB was included in the diagnostic scheme as previous studies have suggested an association between it and TS (Fernando, 1967; Yaryura Tobias *et al*, 1981; Montgomery *et al*, 1982; Nee *et al*, 1982; Frankel *et al*, 1986; Pauls *et*

Table 1
Rates of TS, tics and OCB among first-degree relatives of probands

	Diagnosis									
	TS		CMT		Tics		OCB		TS/Tics/OCB	
	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)
Male relatives ($n=90$)	19	(21.1)	13	(14.4)	2	(2.2)	3	(3.3)	37	(41.1)
Female relatives ($n=78$)	11	(14.1)	8	(10.3)	1	(1.3)	7	(9.0)	27	(35.9)
Total ($n=168$)	30	(17.9)	21	(12.5)	3	(1.9)	10	(6.0)	64	(38.1)

al, 1986; Robertson *et al*, 1988; Robertson & Gourdie, 1990).

The prevalences of TS, CMT and OCB differ with both age and sex. To incorporate these differences into the analyses, separate estimates of prevalence were made. For the first three diagnostic schemes, four fairly narrow age classes (0–5, 6–10, 11–15, and over 15 years) were incorporated. For the analysis that included OCB, four somewhat broader age classes (0–15, 16–25, 26–35, and over 35 years) were used. Furthermore, analyses were carried out using a wide range of age-specific and sex-specific prevalences. Overall population prevalences ranged from 0.00032 to 0.001 for TS only; from 0.005 to 0.030 for TS or CMT; and from 0.008 to 0.05 for TS or CMT/TTD; when OCB was included in the analyses, the overall prevalences ranged from 0.003 to 0.05. The penetrance results are reported only for the accepted prevalence rate (0.5 per 1000; Bruun, 1984), because parameter estimates with the other values proved very similar. Because ascertainment was through consecutive cases, it is likely that the probability of any person with TS in the UK being a proband was quite small. Thus, an ascertainment probability of $\pi=0.01$ was incorporated into the analyses.

Results

Of 168 relatives included in the study (Table 1), 30 (17.9%) had TS, 21 (12.5%) CMT, and 10 (6%) OCB. Consistent with other reports (Pauls *et al*, 1991), males were more likely to have TS or CMT, while females were more likely to receive a diagnosis of OCB.

A wide range of genetic models was examined in a hierarchical fashion. Firstly, the model of no transmission was compared with the mixed model. The mixed model postulates that there is a gene of major effect against a polygenic background that contributes to the manifestation of the disorder. Since there was evidence for vertical transmission in these families (i.e. the model of no transmission could be rejected), additional analyses were done to test specific genetic hypotheses (Table 2). For all the five diagnostic schemes there was evidence that the transmission was consistent with a hypothesis of single-locus transmission. Furthermore, the generalised single-locus model converged to the dominant model for all diagnostic hierarchies. The mixed model converged at the boundary with polygenic heritability (h^2) being zero, and the parameter estimates were essentially identical to the best-fitting Mendelian major-locus model ($d=1$, $t=5.36$, $q=0.0002$, $h^2=0$; and 0% phenocopies for males and females). Furthermore, the mixed model resulted in a significantly better likelihood when compared with the polygenic hypothesis, which therefore could be rejected. There was no evidence to suggest non-Mendelian transmission probabilities. The most parsimonious Mendelian single-locus model was the autosomal dominant model; the estimates of penetrance for this model were 0.966 males and 0.452 for females.

The analyses with the other diagnostic schemes also suggest autosomal dominant transmission. The analysis when subjects with TS or CMT were included gave higher penetrance figures for males (0.999) and females (0.554). When the definition of 'affected' status included those with TS or OCB, the penetrance estimated was 0.882 for males and females. When relatives with TS, tics (CMT/TTD) or OCB were included, the penetrance rate was 0.980 for both sexes (Table 3).

Table 2
Complex segregation analysis on 49 nuclear families (Tourette's syndrome only)

Model of transmission	Diagnostic scheme			
	TS	TS/Tics	TS/OCB	TS/Tics/OCB
No transmission	Rejected***	Rejected***	Rejected***	Rejected***
Polygenic	Rejected**	Rejected*	Rejected***	Rejected**
Mendelian	Consistent	Consistent	Consistent	Consistent
Autosomal dominant	Consistent	Consistent	Consistent	Consistent
Additive	Consistent	Rejected*	Consistent	Consistent
Autosomal recessive	Rejected**	Rejected*	Rejected***	Rejected**

*$P<0.005$, **$P<0.01$, ***$P<0.001$.
Consistent indicates that model of transmission cannot be rejected at $P<0.05$.

Table 3
Genetic model estimates for male and female subjects according to diagnostic schemes

	Prevalence[1]	(Kpm/Kpf)	$p2$	$p1$	$p0$	q
TS only						
male	0.0005		0.9658	0.9658	0.0000	0.0002
female	0.00015		0.4518	0.4518	0.0000	
TS/CMT						
male		0.0029	0.9996	0.9996	0.0001	0.0009
female		0.0010	0.5540	0.5540	0.0000	
TS/tics						
male		0.0030	1.0000	1.0000	0.0001	0.0009
female		0.0010	0.5816	0.5816	0.0000	
TS/OCB						
male		0.0030	0.8818	0.8818	0.0020	0.0009
female		0.0010	0.8818	0.8818	0.0020	
TS/tics/OCB						
male		0.0250	0.9806	0.9806	0.0210	0.0021
female		0.0250	0.9806	0.9806	0.0210	

$p2$, $p1$ and $p0$ denote the penetrance for genotype with two susceptibility alleles (aa), one susceptibility allele (Aa), and no susceptibility allele (AA), respectively, and q the frequency of the susceptibility allele 'a'.
1. Values from Bruun (1984).

Goodness-of-fit tests (χ^2) were carried out for all solutions obtained for all diagnostic schemes. First-degree relatives were grouped according to sex of the proband, sex of the relative, and relationship to the proband (parents v. siblings). Expected risks of being affected with TS, tics or OCB were calculated using the parameters of the autosomal dominant model (the best-fitting model) and compared with the observed rates for all first-degree relatives. For the TS-only scheme, the predicted and the observed frequencies were not significantly different ($\chi^2 = 12.4$, d.f. $= 6$, $0.05 < P < 0.10$). For the TS or CMT group, the predicted rates were significantly different from the observed ($\chi^2 = 20.33$, d.f. $= 6$, $P < 0.005$). Similar findings were obtained when TTD was included with TS and CMT ($\chi^2 = 21.60$, d.f. $= 6$, $P < 0.005$), indicating a poor fit for the data. In the TS/OCB scheme, χ^2 for goodness-of-fit was not statistically significant ($\chi^2 = 3.7934$, d.f. $= 6$, $0.990 < P < 0.9995$), suggesting that OCB is an integral part of the spectrum of expression of TS. Finally, the goodness-of-fit test for TS, CMT/TTD or OCB gave statistically significant values ($\chi^2 = 119.465$, d.f. $= 6$, $P < 0.0005$), again indicating a poor fit for the data. These results suggest that, within these families, motor tics (chronic or transient) may not always be aetiologically or genetically related to TS.

Discussion

We found 17.9% of first-degree relatives to have TS. This is higher than previously reported figures. This may be due in part to referral bias, in that NHNN is a tertiary centre, and families with more than one affected member may be more likely to be referred. It may also be a reflection of a change in trend in the diagnosis of TS over time. It should be noted, however, that even though the rates among first-degree relatives are higher, the distribution of diagnoses is similar to that in other reports (Pauls *et al*, 1991): males are more likely to exhibit TS or tics, and females are more likely to manifest OCB.

The results of the study support the hypothesis that TS is inherited as an autosomal dominant trait with high penetrance. Furthermore, the results are consistent with the hypothesis that OCB is part of the spectrum of the syndrome. The best fit for the data was obtained when OCB was also included.

In order to allow comparison with previous studies, data were analysed using the higher prevalence rates as assumed by Comings *et al* (1984) and Devor (1984). This did not alter the inferences; all results remained consistent with autosomal dominant transmission. Although segregation analysis suggested an autosomal dominant mode of transmission for all diagnostic schemes, the estimated values did not correspond with the observed rates in the relatives for those schemes including tic disorders. The difference was most marked in the rates for mothers of male probands, where the observed rate was much higher than that expected. The rate for fathers showed a more slight but similar trend. This should be viewed in the context of the fact that the sex ratio distribution of affected relatives in our data is different from that in other published studies, with more females being affected than expected. Thus the rates do not conform with the sex ratio of population prevalences used in the analyses, which may be contributing to the goodness-of-fit results. However, a more recent epidemiological study (Apter *et al*, 1992) has shown a male : female ratio of approximately 1.6 : 1, which is similar to our findings.

It is interesting to note that the estimated values compared best with the observed values for the TS and OCB group. This is consistent with a report by Pauls & Leckman (1986) on an independent US sample suggesting that obsessive–compulsive disorder (OCD) is aetiologically and genetically related to TS. On the other hand, our results suggest that not all chronic tics may be related to TS. When goodness-of-fit χ^2 were calculated, the analyses that included CMT category resulted in the most significant differences between observed and expected. This suggests that either CMT is related to TS and the genetic model is wrong, or that some individuals with CMT do not have a disorder that is related to TS, and that, within these families, motor tics (chronic and transient) are phenocopies.

To help understand the relationship between OCB, CMT and TS, phenomenological studies using personal interview to assess different expressions of the syndrome are indicated. It also emerges that sound epidemiological studies are of crucial importance at this juncture to examine some of the issues raised here, particularly that of true estimates of the prevalence of TS in the general population, sex ratio, and sex-dependent differences in the expression of the disorder.

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A microsatellite polymorphism at the THRB locus

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Source/Description: A human genomic library in EMBL3 (Clontech) was screened with a human repeat free fragment probe of pBH302 at locus THRB (1). Chromosome walking identified a clone, lambda THRB-5. One AluI fragment of lambda THRB-5, subcloned in M13, hybridised to poly(dC-dA). Sequencing of this subclone identified a $(GT)_n$ repeat which detects 5 alleles. The subclone was designated THRB-5GT. Flanking sequences were used to design PCR primers to amplify the repeat sequence.

PCR Primers:

THRB-5 1 5'-AACTGACTCTACTGACACCTG-3'
THRB-5 2 5'-ATGGTACCCTCATTCCTTAGG-3'

Frequency: Estimated from 42 chromosomes of unrelated European Caucasians.

THRB-5GT

Allele	Size (nt)	Frequency
A1	201	0.07
A2	195	0.08
A3	193	0.33
A4	191	0.42
A5	189	0.10

Heterozygosity = 0.66

Chromosomal Localisation: THRB formerly the ERBA2 locus, Douglas *et al.* (2), has been mapped to chromosome 3 by Drabkin *et al.* (3) to 3p21.33−p22. Microsatellite probe localised to THRB by linkage to the RFLP.

Mendelian Inheritance: Co-dominant segregation was observed in 6 two or three generation families.

PCR Conditions: PCR is performed in 12.5 μl containing: 50 ng DNA, 12.5 pmoles of each primer, 1.0 mM $MgCl_2$, 10 mM Tris-Cl pH 8.3, 50 mM KCl, 25 μM dATP and 200 μM of other dNTPs, 1 unit Taq polymerase (Perkin-Elmer/Cetus), 0.01% gelatin and 0.5 μl of ^{35}S-dATP at 500 Ci/mmol (Dupont). Amplification is for 35 cycles with denaturation at 94°C, annealing at 57°C and extension at 72°C, all for 1 minute each. The dinucleotide repeat sequenced was $(GT)_4$ (A) $(GT)_6$ $(G)_{13}$ $(GT)_3$, and is different from the sequence reported by Sakurai *et al.* (4).

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Genomic cloning and localization by FISH and linkage analysis of the human gene encoding the primary subunit NMDAR1 (GRIN1) of the NMDA receptor channel

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SUMMARY

A cDNA clone of the NMDAR1 (isoform E) has been used to screen both lambda and cosmid genomic libraries. A genomic phage clone was identified and sequenced and was found to contain some of the 3′ coding regions of the GRIN1 gene. This clone was used to localize the gene using fluorescent *in situ* hybridization (FISH) to normal chromosomes, and also to a lymphoblastoid cell line containing a translocation involving chromosomes 9 and 15. FISH localized the gene to chromosome 9q34.3. The clone was used to screen a panel of genomic DNAs cut with 20 restriction enzymes. A VNTR sequence 5′ to the gene, which was polymorphic for a number of restriction enzymes, was detected. A PvuII fragment of the genomic clone was found to detect the VNTR on Southern hybridization. The polymorphic VNTR marker was mapped against chromosome 9q34 markers using linkage analysis in the CEPH families. The GRIN1 gene was linked to D9S7 with a maximum lod score of 20·09 at zero recombination fraction in males and 0·03% recombination in females.

INTRODUCTION

The glutamate receptors consist of two main classes, ionotropic and metabotropic. The metabotropic receptors are coupled to intracellular signal transduction through G-proteins (Schoepp *et al.* 1990). The ionotropic receptors contain integral cation-specific ion channels and are divided into: NMDA (N-methyl D-aspartate) receptors; AMPA (alpha)3-hydroxyl-5-methyl-4-isoxazoleproprionicacid) receptors, and kainate receptors (Monaghan *et al.* 1989). The NMDA (N-methyl-D-aspartate) receptor is involved in memory and the mediation of excitotoxicity. There are 5 cloned subunits of the NMDA receptor; NMDAR1 (which has 7 isoforms A–G) and is designated GRIN1, and NMDAR2A, 2B, 2C, 2D. The functional receptor is a heteromer consisting of NMDAR1, which confers the NMDA properties of the receptor combined with the NMDAR2 subunits.

Moriyoshi *et al.* (1991) demonstrated that NMDAR1 is the key subunit of the receptor as it exhibits the pharmacological and electrophysiological properties of the NMDA receptor. In the rat there are seven isoforms of NMDAR1 (A–G) formed by alternative splicing. The other constitutive subunits NMDAR2 (A–D) do not exhibit the intrinsic NMDA receptor properties, but have the ability to potentiate NMDAR1 activity (Kutsuwada *et al.* 1992). Functional NMDA receptors are generated by the co-expression in transfected cells or *Xenopus* oocytes of the NMDAR1 subunit with specific members of the NMDAR2 subunit family conferring functional variability in the electrophysiological and pharmacological properties of the receptors (Moriyoshi *et al.* 1991; Kutsuwada *et al.* 1992). It is thought that *in vivo* the different spatial and temporal expression of these subunit genes results in the generation of NR1 and NR2 heteromeric receptor subtypes (Kutsuwada *et al.* 1992).

The NMDA receptor mediates many of the excitatory functions of glutamate, including long-term potentiation and long-term depression; these long-term changes in neuronal responsiveness could explain certain aspects of learning and memory (Collingridge *et al.* 1990; Monaghan *et al.* 1989). Additionally, in pathological situations, the excessive activation of excitatory synapses has been hypothesized to cause neuronal death in neurodegenerative disorders due to excitotoxicity (Choi, 1991). The excitotoxic effect is mediated by abnormal increases in intraneuronal calcium ions. In order to study this gene in human disorders it is helpful to identify polymorphic genetic markers within or near the NMDAR1 gene.

A cDNA clone of the NMDAR1 (Le Bourdelles *et al.* 1993) gene was therefore used to screen a genomic phage library and 3 different genomic cosmid libraries in order to find a polymorphic marker for the locus. This screening yielded only 1 positive clone from the phage library, supporting the observations of others about the inherent cloning difficulties of this gene (Henneberry, 1992). The clone (AD1) was used for FISH on both normal chromosomes and also on the lymphoblastoid cell line B00015 (described in Povey *et al.* 1992 and obtained from the European Cell Culture Repository, Wilts) which has the karyotype 46XXt(9;15)(q34.3;q24). The breakpoint on chromosome 9 has previously been shown to be distal to D9S14 and RXRA (Zhou *et al.* 1992) and we have recently determined that it is distal to D9S114 (K. Woodward, personal communication, 1993, data not shown). AD1 was screened for simple dinucleotide repeats using poly(dC.dA).(dG.dT) (Pharmacia), and for restriction length polymorphisms (RFLPs) using the radiolabelled AD1 on Southern blots restricted with 20 different enzymes. PCR sequencing was performed on a 2·3 kb subclone called PMB3.

MATERIALS AND METHODS

Hybridization of genomic lambda libraries

The library was titred in a suitable host (LE392 for Clontech EMBL3 Sau3A1 partial HL1067j genomic library). 30000 phage were plated out per 15 cm petri dish, and a total of 20 plates were prepared such that 4–5 genomes were screened (Clarke & Carbon, 1976). The phage were then screened using the method described by Sambrook *et al.* (1989).

Hybridization of cosmid genomic libraries

Three human cosmid libraries were screened using the protocol described by Kiousis *et al.* (1987).

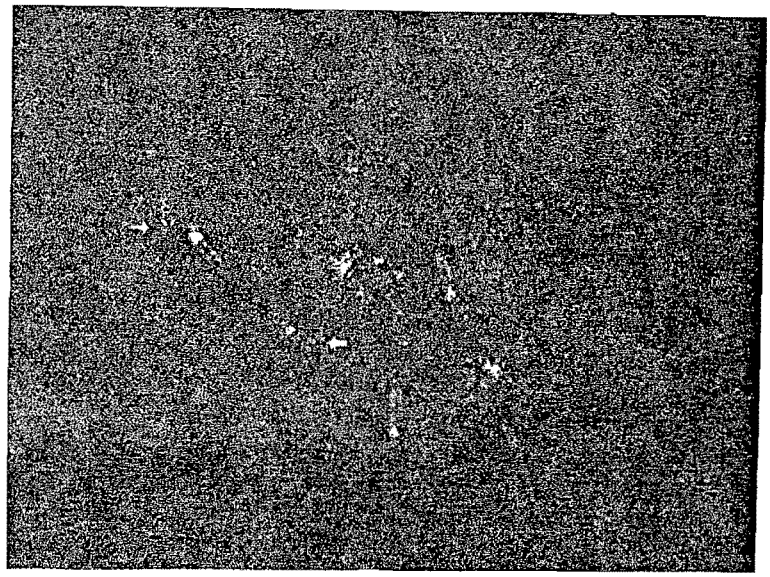

Fig. 1. Hybridization of a genomic NMDAR1 clone by FISH on the lymphoblastoid cell line B00015 (karyotype 46XXt(9;15)(q34.3;q24)), showing the presence of the cloned sequence on the translocated chromosome 15 and normal chromosome 9.

DNA preparation, digestion and Southern hybridization

DNA was isolated from peripheral lymphocytes (Sambrook *et al.* 1989). Five to 10 μg DNA was digested with 20 to 30 units of restriction enzymes overnight in buffers recommended by the manufacturer (Boehringer Mannheim or Amersham). The DNA was electrophoretically separated on 0·8% agarose gels and transferred to a nylon membrane (Hybond^N, Amersham) according to the manufacturers' protocols. The DNA was fixed to the membrane by UV irradiation. Prior to hybridization the blots were prehybridized in 0·9 M-NaCl, 1% SDS with sheared salmon sperm DNA at a final concentration of 50 mg/ml for at least 4 hours. Plasmid DNA from the probes was isolated according to the alkaline lysate procedure of Birnboim & Doly (1979). Insert DNA was prepared by electroelution or by separation in low-melting agarose (Sambrook *et al.* 1989). The insert DNA was labelled by random priming (Feinberg & Vogelstein, 1983) with ^{32}P-dCTP (Amersham) to a specific activity of $2–10 \times 10^8$ cpm/μg and, after denaturation, was incubated for 1 hour at 65 °C with COT1 DNA (Gibco BRL Cat no. 5279SA) to compete-out the common repeat sequence before being added to the hybridization buffer (0·9 M-NaCl, 1% SDS, 10% dextran sulphate, 50 mg/ml sheared salmon sperm DNA). Hybridization was at 65 °C overnight. The blots were washed once in 1 × SSC, 0·1% SDS for 20 min at 65 °C, followed by two washes at 65 °C in 0·25–0·1 SSC, 0·1% SDS for 20 min each. The hybridized filters were autoradiographed using Fuji X-ray film at −70 °C.

Subcloning and Pcr sequencing

The PvuII 2·3 kb fragment PMB3 was subcloned using the pCR-Script SK(+) cloning kit from Stratagene Cat no. 211190. The sequencing was performed using the fmol DNA sequencing system from Promega Cat no. TM024.

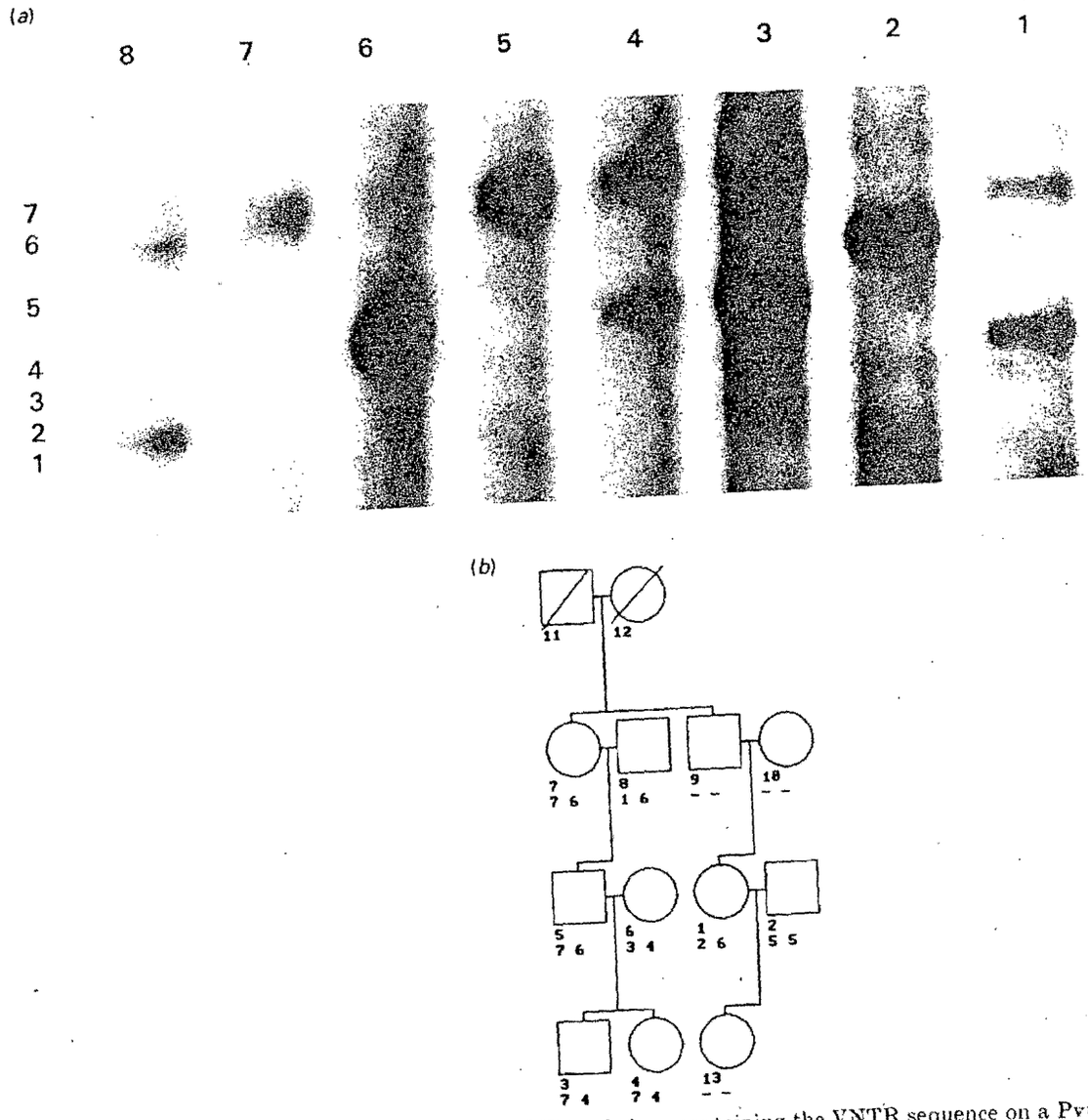

Fig. 2. (*a*) Southern hybridization of the PMB3 subclone containing the VNTR sequence on a PvuII filter of a small nuclear family showing 7 alleles. (*b*) The genotypes are shown on the pedigree diagram.

Fluorescent in situ hybridization

The FISH was performed on both normal chromosomes and the lymphoblastoid cell line B00015 using the method described by Fitzgibbon *et al.* (1993).

Linkage

Linkage was performed using the CRI-MAP (Donis-Keller *et al.* 1987) computer programme.

RESULTS

Figure 1 shows that GRIN1 maps to the derivative chromosome 15, distal to the breakpoint and therefore lies distal to D9S114. A variable numbered tandem repeat (VNTR) was identified

Table 1. *Two point lodscore table (female above and male below) for NMDAR1 and chromosome 9q markers. (b) Maximum likelihood estimates of recombination fraction (theta) and the corresponding value of Z max*

(The order of markers shown in both tables is centromere to qter. D9S11 is the most distal marker in the chromosome 9 consortium map (Attwood *et al.* 1994). Those markers shown on the same line do not recombine in the CEPH data and were treated as one during the analysis.)

(a)

Theta:	0·001	0·05	0·10	0·20	0·30	0·40
DBH/D9S66	−1·94	6·87	7·60	6·95	5·15	2·65
	−9·44	4·67	6·14	6·09	4·53	2·21
D9S14/D9S67	4·97	6·78	6·61	5·40	3·62	1·50
	5·72	8·21	7·82	6·27	4·20	1·80
D9S17	4·28	5·49	5·27	4·33	3·03	1·48
	1·24	2·73	2·75	2·29	1·54	0·66
D9S7	5·11	6·20	5·84	4·71	3·27	1·61
	13·83	12·78	11·65	9·19	6·42	3·29
D9S11	3·53	7·61	7·53	6·31	4·47	2·20
	11·02	10·30	9·44	7·49	5·14	2·52

(b)

	θf	θm	Z max
DBH/D9S66	0·11	0·14	14·01
D9S14/D9S67	0·06	0·05	15·00
D9S17	0·04	0·07	8·28
D9S7	0·03	0·00	20·09
D9S11	0·07	0·00	18·69

with over 12 alleles (Figure 2) ranging in size from 2·1 kb to 4·5 kb, which were difficult to read due to the presence of non-specific repeat sequences. The VNTR was not found in the cDNA clone, demonstrating that the polymorphic sequence is located in an intron or at the 3′ or 5′ ends of the gene. A 2·3 kb PvuII fragment of AD1 was found to contain the VNTR sequence and was used to hybridize against the CEPH reference family PvuII filters. Linkage analysis was performed; the polymorphism gave a maximum lod score of 20·09 at $\text{theta}_{m,f}$ (0·00 and 0·03) with D9S7 (Table 1). It could not be precisely positioned on the CEPH chromosome 9 consortium map (Attwood *et al.* 1994) at odds of 1000:1 but could be confidently placed distal to D9S67. The 2·3 kb PvuII fragment containing the VNTR was subcloned into the pCR-Script™ SK (+) phagemid (Stratagene) and called PMB3 in an attempt to remove the non-specific repeat sequences and make hybridization more specific, however, this did not make the signals from Southern hybridizations easier to read. Some of the sequence was determined and analysed using the BLAST program (Altschul *et al.* 1990). 60 bases of the sequence obtained showed 100% homology to the 3′ end of the published mRNA sequence for the human NMDAR1 subunit (Karp *et al.* 1993), 94% with rat (Moriyoshi *et al.* 1991) and 93% with mouse (Yamazaki *et al.* 1992).

DISCUSSION

While this work was in progress, two other groups have reported assignment of GRIN1 to 9q34.3 (Collins *et al.* 1993; Karp *et al.* 1993) using FISH, mentioning the possibility that GRIN1 might be a candidate locus either for Tuberous sclerosis (TSC1) or for Idiopathic Torsion Dystonia, both mapping to 9q34. However, we have shown both by physical mapping and by genetic means, that GRIN1 lies distal to the distal flanking markers for these diseases

(Kwiatkowski *et al.* 1993) and can therefore be excluded as a candidate gene. The distal position of GRIN1 makes it a valuable genetic marker for 9qter and we are currently attempting to format the marker for use with PCR.

This work was supported by MRC project grant G9023197N and also grants from the USA Tourette Syndrome Association, the UK Tourette Syndrome Association and the Clinical Research and Development Committee of the Middlesex Hospital. J. A. and S. P. acknowledge the support of the MRC as part of the Human Genome Mapping Project. The BLAST search was done using the facilities at the MRC HGMP Resource Centre. We acknowledge the support of Eurogem and of CEPH. K. W. is supported by an HGMP MRC studentship.

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Exclusion of the 5-HT_{1A} Serotonin Neuroreceptor and Tryptophan Oxygenase Genes in a Large British Kindred Multiply Affected With Tourette's Syndrome, Chronic Motor Tics, and Obsessive-Compulsive Behavior

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Objective: *Previous studies have demonstrated a relationship between obsessive-compulsive disorder or behavior and Gilles de la Tourette syndrome. It has been hypothesized that the serotonergic system is implicated in the etiology of obsessive-compulsive disorder. Therefore, the authors investigated whether genetic variation in a serotonergic receptor and a modifying enzyme were associated with Tourette's syndrome.* *Method:* *A linkage analysis using DNA and blood group markers was carried out in a large British kindred multiply affected with Tourette's syndrome, chronic motor tics, and obsessive-compulsive behavior.* *Results:* *There was no evidence to support the hypothesis that genetic variation in the serotonin 5-HT_{1A} receptor and tryptophan oxygenase genes causes susceptibility to Tourette's syndrome and chronic multiple tics.* *Conclusions:* *The results eliminate two possible candidate genes from having a role in the pathophysiology of Tourette's syndrome.*

(Am J Psychiatry 1995; 152:437–440)

The association between Gilles de la Tourette syndrome and obsessive-compulsive disorder is well established, with evidence from historical, epidemiological, phenomenological, and genetic research. This association has been fully reviewed by Robertson and Yakeley (1). Neuroendocrine studies suggest that the function of serotonin 5-HT_1 receptors (particularly, 5-HT_{1A}) may be disturbed in obsessive-compulsive disorder (2); however, the exact nature of the alterations in serotonergic function is uncertain. There have also been descriptions of abnormalities in serotonin metabolism in Tourette's syndrome in which CSF levels of 5-hydroxyindoleacetic acid are decreased after probenecid loading (3). It has been suggested that these changes in patients with Tourette's syndrome are due to a mutation in the tryptophan oxygenase gene (4).

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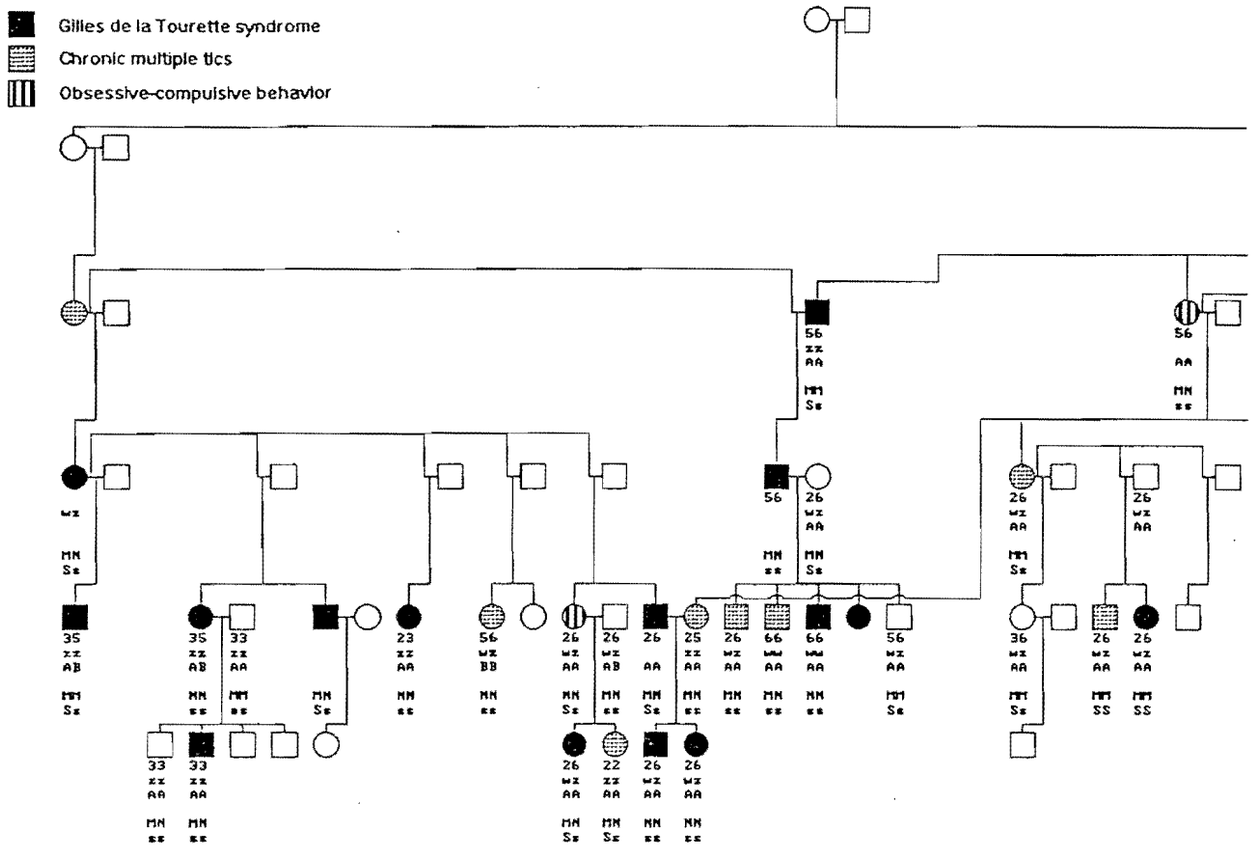
METHOD

The pedigree we studied consists of 116 members (figure 1), 85 of whom were individually interviewed. Of the 85 interviewed, 29 had Tourette's syndrome, a further 20 had chronic multiple tics without Tourette's syndrome, and five had obsessive-compulsive behavior alone; these clinical findings have been fully documented (5). The structure and paternity of the kindred were confirmed with the use of DNA fingerprinting.

Patients were assigned to diagnostic categories as follows: 1) definite or probable Tourette's syndrome, diagnosis according to the DSM-III criteria by history and/or examination; 2) definite or probable chronic multiple tics, diagnosis according to DSM-III by history

FIGURE 1. A Large Kindred, Multiply Affected With Tourette's Syndrome, Chronic Multiple Tics, and Obsessive-Compulsive Behavior, Used for Linkage Analysis, Showing the Genotypes Analyzed[a]



[a]A square indicates a male; a circle indicates a female. Alleles are shown in the following order: P599GT$_{(n)}$ (topmost marker), L599Ha, G-21, and MN and Ss blood groups.

TABLE 1. Linkage Markers, Their Locations, and Allele Frequencies Used in a Study of Members of a Kindred With Tourette's Syndrome and Chronic Multiple Tics

Probe	Locus	Chromosomal Location	Allele	Frequency
G-21	HTR_{1A}	5cen-q11	A1	0.78
			A2	0.22
L599Ha	D5S76	5q11.2	A1	0.32
			A2	0.16
			A3	0.50
			A4	0.02
P599GT$_{(n)}$	D5S76	5q11.2	A1	0.021
			A2	0.213
			A3	0.213
			A4	0.127
			A5	0.064
			A6	0.362
MN and Ss blood groups	MNS	4q31	M	0.53
			N	0.47
			S	0.31
			s	0.69

and/or examination; and 3) obsessive-compulsive behavior without Tourette's syndrome or chronic multiple tics, diagnosis by history and, in the case of children, corroboration by parents.

Restriction fragment length polymorphism (RFLP) and microsatellite polymorphism were determined with the use of the protocols set out by Melmer et al. (6) and Sherrington et al. (7). On the basis of the results of a previous segregation analysis (8), autosomal transmission was assumed with a gene frequency of 0.0005 and heterozygote penetrances of 0.50 for Tourette's syndrome and 0.88 for Tourette's syndrome and chronic multiple tics. To allow for occasional phenocopies, the normal homozygote penetrance was set at 0.001. Two diagnostic categories were used to indicate affected status: Tourette's syndrome only and Tourette's syndrome and chronic multiple tics. No separate analysis was carried out in which the cases of obsessive-compulsive behavior were included as affected because four of the five individuals with obsessive-compulsive behavior were obligate carriers under the assumption of dominant transmission. The MLINK program of the LINKAGE package of programs (9) was used to carry out linkage analysis between markers and different affection classes.

From published linkage data (6) the 5-HT_{1A} locus (HTR_{1A}) maps to within 2 cM of the locus D5S79 on chromosome 5. Therefore, markers at this locus can be used to exclude 5-HT_{1A} if sufficient exclusion data are obtained from the linkage analysis. Two markers were used, an RFLP (L599Ha) and a microsatellite polymorphism (P599GT$_{(n)}$) (6, 7). The tryptophan oxygenase gene is less than 1 cM in distance from the MNS blood group (4); therefore, this locus was used as a marker for the tryprophan oxygenase gene itself. For the MNS blood group, two allelic systems (MN and Ss) were defined with zero recombination between them. The locations and allele frequencies of the markers that were used are listed in table 1. When linkage

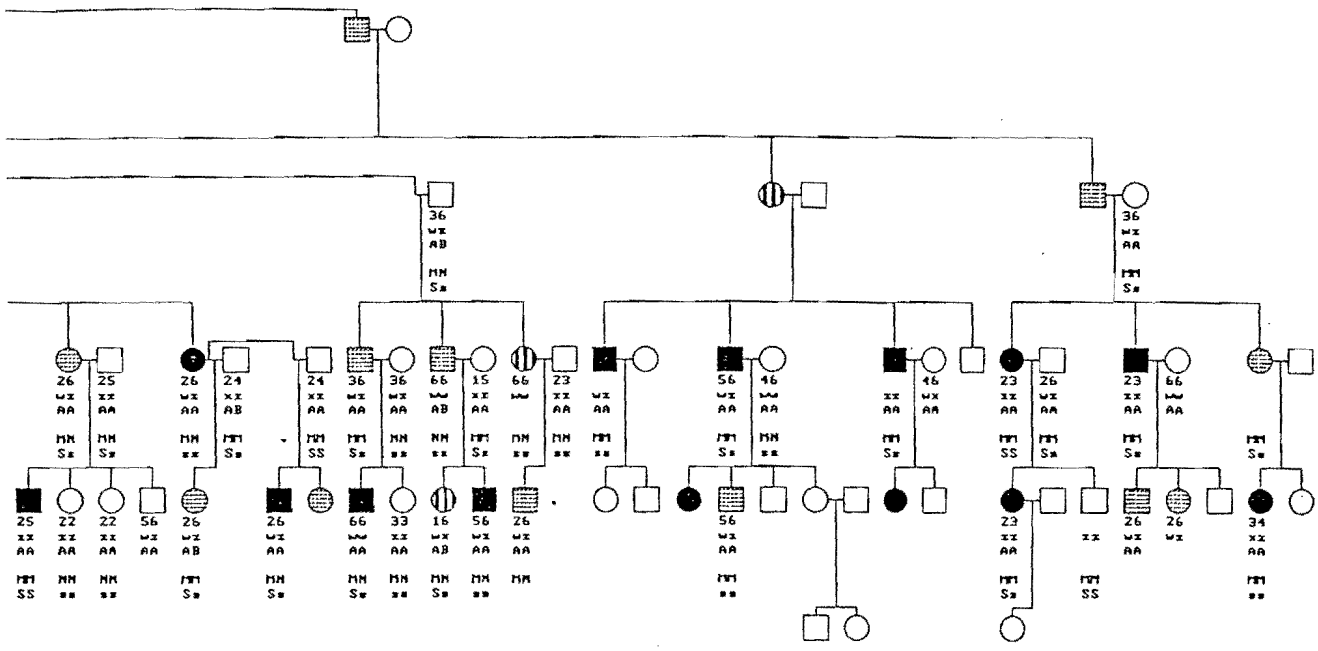


TABLE 2. Two-Point and Three-Point Lod Scores Between Affection Class and Genetic Markers for Diagnostic Models of Tourette's Syndrome Alone and Tourette's Syndrome and Chronic Multiple Tics

Markers and Diagnosis	Lod Score for Each Recombination Fraction (θ)							
	θ=0.000	θ=0.001	θ=0.010	θ=0.050	θ=0.100	θ=0.200	θ=0.300	θ=0.400
Two-point lod score: G-21 TaqI RFLP[a]								
Tourette's syndrome	0.908	0.905	0.884	0.793	0.685	0.492	0.320	0.159
Tourette's syndrome and chronic multiple tics	−2.000	−1.751	−1.057	−0.398	−0.111	0.133	0.200	0.153
Three-point lod scores								
L599Ha RFLP and P599GT$_{(n)}$ microsatellite at D5S76								
Tourette's syndrome	−3.904	−3.596	−2.531	−1.522	−0.999	−0.413	0.025	0.131
Tourette's syndrome and chronic multiple tics	−14.980	−13.591	−10.160	−5.646	−2.835	−0.421	0.341	0.365
Blood groups MN and Ss on chromosome 4								
Tourette's syndrome	−12.365	−11.975	−9.855	−6.152	−4.086	−1.958	−0.938	−0.385
Tourette's syndrome and chronic multiple tics	−22.059	−19.349	−14.323	−9.208	−6.798	−3.986	−2.147	−0.864

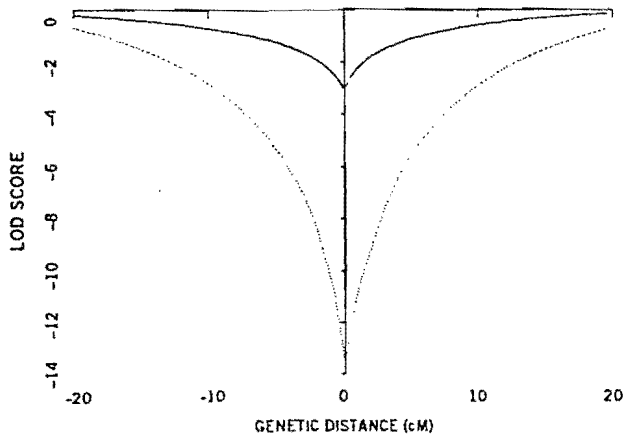
[a]RFLP=restriction fragment length polymorphism.

analysis was carried out between the RFLP and the microsatellite polymorphism at D5S76, the maximum lod score of 8.660 was at a recombination fraction of zero. The two-point lod scores for D5S76 (RFLP and microsatellite) and G-21 were combined into an overall map with the use of FASTMAP, a program that combines lod scores on a fixed map, taking into account the number of informative meioses for each marker (10).

RESULTS

The 5-HT$_{1A}$ probe G-21 gave results that were only weakly informative and detected no recombinants with Tourette's syndrome. However, when cases of chronic multiple tics were also included as affected, a lod score

FIGURE 2. Estimated Multipoint Lod Scores Produced by the FASTMAP Program on the Basis of Two-Point Lod Scores at D5S76 and 5-HTR_{1A} Loci of Members of a Kindred for Tourette's Syndrome Alone and Tourette's Syndrome and Chronic Multiple Tics[a]



[a]The solid line represents lod scores for the Tourette's syndrome diagnostic model, and the dotted line the scores for Tourette's syndrome and chronic multiple tics. D5S76 has been placed at a map position of 0 cM, and 5-HTR_{1A} at 2 cM.

of −2.000 at zero recombination was obtained (table 2). Counting only cases of Tourette's syndrome as affected resulted in an exclusion (lod less than −2.000) up to a recombination fraction of 1% with D5S76, and when cases of chronic multiple tics were also included, an exclusion of over 10% was achieved (table 2). FASTMAP (figure 2) produced good exclusions for both models. This certainly excludes all of the region to which the 5-HT_{1A} gene has been mapped. With the MNS blood group, an exclusion of nearly 20% was obtained for Tourette's syndrome, and an exclusion of over 30% for chronic multiple tics (table 2).

DISCUSSION

Our analysis eliminates one possible hypothesis about the pathophysiology of Tourette's syndrome in one very large multiplex kindred when an autosomal dominant mode of transmission is assumed. The fact that considerable effort has been made in linkage analysis of Tourette's syndrome and that currently about 85% of the genome has been excluded implies either that a susceptibility locus will shortly be identified or that there is genetic heterogeneity for the syndrome. If heterogeneity is present, then clearly, single large kindreds such as ours and those reported in Canada, Oregon, and Utah (5, 11) should be studied independently. Data across studies should not necessarily be used to produce combined exclusion maps.

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The Genetic Susceptibility to Gilles de la Tourette Syndrome in a Large Multiple Affected British Kindred: Linkage Analysis Excludes a Role for the Genes Coding for Dopamine D1, D2, D3, D4, D5 Receptors, Dopamine Beta Hydroxylase, Tyrosinase, and Tyrosine Hydroxylase

Peter M. Brett, David Curtis, Mary M. Robertson, and Hugh M.D. Gurling

Segregation analyses have shown that Gilles de la Tourette Syndrome (GTS) is transmitted as an autosomal dominant gene disorder indicating that classical linkage analysis should be able to identify susceptibility loci. Previous studies of GTS have included investigations of neuroreceptor function, neurotransmitters, and their metabolites as well as neurotransmitter-related enzymes in an attempt to determine the pathophysiology of GTS. The neurotransmitter systems most often thought to be involved in GTS include those involving adrenaline, noradrenaline, and dopamine. We have carried out research to test the hypothesis that genes encoding proteins in the catecholamine pathways may contribute to the genetic etiology of GTS. Polymorphic markers at or near the D1, D2, D3, D4, D5 neuroreceptor gene loci as well as at the genes encoding dopamine beta hydroxylase (DBH), tyrosinase (TY) and tyrosine hydroxylase (TH) were studied in one large multiple affected pedigree. The linkage results of this investigation exclude a major role of these candidate genes in the etiology of GTS in the pedigree.

Key Words: Tourette syndrome, linkage analysis, dopamine receptor

Introduction

Gilles de la Tourette Syndrome (GTS), first described in 1825 by Itard and later in 1885 by Georges Gilles de la Tourette is characterized by multiple motor and one or more vocal tics (American Psychiatric Association 1987; World Health Organization 1992). The syndrome, as originally described, was thought to be rare but more recent work has shown that GTS and the genetically related disorder chronic motor tics (CMT) may have a life time prevalance of at least 0.5 per thousand (Robertson 1989). GTS is thought to be genetically determined with the mode of transmission being autosomal dominant with incomplete penetrance as shown by segregation analyses carried out on independent GTS samples (Comings et al 1984; Devor 1984; Price et al 1984;

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Pauls and Leckmann 1986; Curtis et al 1992; Eapen et al 1993). Several twin studies have given evidence of variable phenotypic expression (pleiotropy) for the genetic susceptibility to GTS (Shapiro and Shapiro 1980; Jenkins and Ashby 1983; Waserman et al 1983; Price et al 1985).

A possible link between the catecholamine system and GTS has been reviewed by Caine (1985). Observations on the treatment of GTS patients have provided the most evidence in favor of a role for the dopamine system in the etiology of GTS. Successful treatments used to ameliorate the symptoms of GTS employ predominantly D2 receptor antagonists such as haloperidol (Shapiro and Shapiro 1982), pimozide (Golden 1984) and sulpiride (Robertson et al 1990). There are now five recognized dopamine receptor genes that have been cloned and localized. It has been shown that commonly used dopamine antagonists have affinity for all five dopamine receptors (van Tol et al 1992), but that affinity for subtypes varies considerably. In addition, it is known that dopamine antagonists have multiple actions on other brain transmission systems (Gilman et al 1992; Baldessarini 1980).

The effects of dopaminergic agonists, such as L-DOPA (Sacks 1982), and central nervous system (CNS) stimulants, such as methylphenidate (Golden 1984; Robertson and Eapen 1992), are deleterious and increase the number and severity of the symptoms shown by GTS patients, whereas the dopamine depletors, such as tetrabenazine (Jankovic et al 1984), do induce some improvement in the symptoms. These drugs provide evidence of the possible involvement of the enzymes controlling the synthesis and breakdown of dopamine. Dopamine beta hydroxylase (DBH) catalyses the conversion of dopamine to norepinephrine in the adrenal medulla (Kirshner 1957). Tyrosine hydroxylase (TH) is the first enzyme in the catecholamine biosynthetic pathway and is responsible for the conversion of L-tyrosine to 3,4-dihydrophenylalanine (DOPA) (Cooper et al 1991). Tyrosinase is involved in the conversion of tyrosine to DOPA to be used in the synthesis of melanin (Cooper et al 1991). These genes are, therefore, essential in the functioning of the catecholaminergic pathways and a mutation in any one of them could have an impact on overall catecholamine metabolism.

It is however not clear from the evidence whether the catecholaminergic system itself is in some way etiologically responsible for GTS, or whether the changes observed are the effect of another brain system dysfunction. Bornstein and Baker (1990) measured changes in several aminergic systems in a controlled study. Significant decreases in beta-phenylethylamine (PEA), 5-hydroxytryptamine, p-tyramine and 3-methoxy-4-hydroxyphenylglycol were found in patients' urine. After control for the effects of drugs the most pronounced change was found in PEA. PEA is produced by the decarboxylation of phenylalanine and has a mescaline-related psychotogenic action (Cooper et al 1991). It is known that PEA has effects on brain dopamine, 5-HT and norepinephrine (Jackson and Smythe 1973; Sloviter et al 1980).

Methods

Family Collection and Diagnosis

The pedigree was identified when the proband presented at a specialist GTS clinic. The pedigree studied consists of 116 members (Figure 1), 85 of whom were individually interviewed. The structure and paternity of the kindred was confirmed using deoxyribose nucleic acid (DNA) fingerprinting.

One of us (MMR) evaluated individuals in the pedigree. Subjects were interviewed to assess "caseness" using a semistructured diagnostic interview to obtain DSM III criteria for GTS and chronic multiple tic syndrome (CMT), which has been developed by MMR (Robertson et al 1988). The protocol used is highly comparable with other workers in the field. Other psychiatric diagnoses were made using the SADS-L standardized interview (Spitzer and Endicott 1977) and Research Diagnostic Criteria (RDC) (Spitzer et al 1978).

Patients were assigned to diagnostic categories as follows:

1. Definite or probable GTS; satisfying DSM III (American Psychiatric Association 1980) criteria on history and/or examination.
2. Definite or probable CMT; DSM III diagnosis on history and/or examination.
3. Obsessive compulsive behaviors (OCB) without GTS or CMT; obtained on history and, in the case of children, corroborated by parents.

Of the 85 interviewed 29 had GTS, a further 20 had CMT without GTS and 5 had OC behaviors alone (Robertson and Gourdie 1990).

RFLP and Microsatellite Polymorphism Determination

DNA was isolated from peripheral lymphocytes (Sambrook et al 1989). Five to 10 μg DNA was digested with 20 to 30 units of restriction enzymes overnight to buffers recommended by the manufacturer (Boehringer Mannheim or Amersham). The DNA was electrophoretically separated on 0.8% agarose gels and transferred to a nylon membrane (Hybond N, Amersham, U.K.) according to the manufacturers' protocols. The DNA was fixed to the membrane by UV irradiation. Prior to hybridization the blots were prehybridized in 0.9M NaCl, 1% SDS with sheared salmon sperm DNA at a final concentration of 50 mg/ml for at least 4 hr. Plasmid DNA from the probes was isolated according to the alkaline lysate procedure of Birnboim and Doly (1979).

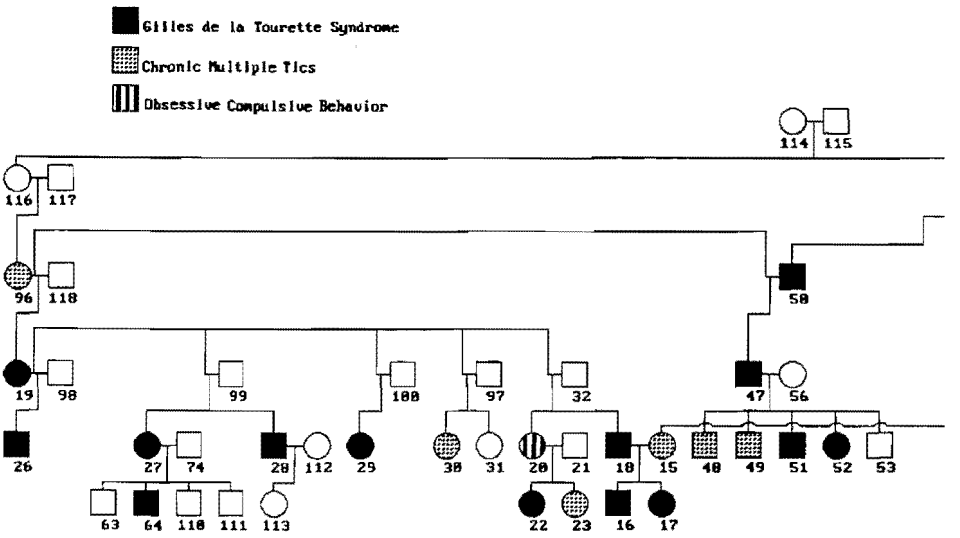

Figure 1. Multiplex GTS/CMT Kindred (F24) studied by linkage analysis.

Insert DNA was prepared by electroelution or by separation in low-melting agarose (Sambrook et al 1989). The insert DNA was labelled by random priming (Feinberg and Vogelstein 1983) with ^{32}P-dCTP (Amersham, U.K.) to a specific activity of 2-10 x 10^8 cpm/μg and, after denaturation, added to the hybridization buffer (0.9M NaCl, 1% SDS, 10% Dextran Sulfate, 50 mg/ml sheared salmon sperm DNA). Hybridization was at 65° C overnight. The blots were washed once in 1×SSC, 0.1% SDS for 20 min at 65° C, followed by two washes at 65° C in 0.5 to 0.1 SSC, 0.1% SDS (the stringency varied depending on the probe being used) for 20 min each. The hybridized filters were autoradiographed for up to 14 days using Fuji X-ray film at −70° C.

Polymerase chain reaction (PCR) was performed in 12.5 μl containing: 50 ng DNA, 12.5 pmoles of each primer, 1.0 mmol/L $MgCl_2$, 10 mmol/L Tris-Cl pH 8.3, 50 mmol/L KCl, 25 μmol/L dATP and 200 μmol/L of other dNTPs, 1 unit Taq polymerase (Perkin-Elmer/Cetus), 0.01% gelatin and 0.5 μl of ^{35}S-dATP at 500 Ci/mmol (Dupont, St. Louis, MO). End-labeled primers were also used for some microsatellite amplifications (100 pmoles of primer labeled in 10μl with 1 μl gamma-^{32}P ATP at 3000 Ci/mmol, 0.1 μl of this was used per reaction). Amplification was for 35 cycles with denaturation at 94° C, annealing at the appropriate temperature for the primers and 20 sec extension at 72° C. The amplified product was separated by polyacrylamide gel electrophoresis using a urea denaturing gel. The gels were fixed in 10% acetic acid/10% methanol and vacuum dried onto 3 mm paper. The gels were autoradiographed using Fuji X-ray film at −70° C for 1 hr to 2 days depending on the primers used.

Linkage Analysis

Based on the results of a previous segregation analysis (Curtis et al 1992) autosomal transmission was assumed with a gene frequency of 0.0005, and heterozygote penetrances of 0.5 for GTS and 0.88 for GTS and CMT. To allow for occasional phenocopies, the normal homozygote penetrance was set to 0.001. Two diagnostic categories were used to indicate positive affection status, GTS only and GTS and CMT. No separate analysis was carried out including the OCB cases as affected, because four out of five of the OCB cases were obligate carriers under the assumption of dominant transmission. Six of the nine markers used had six or more alleles, these were condensed to just five alleles for the purpose of the linkage analysis. This may lead to a slight loss of information in some cases and so may result in some reduction of the overall magnitude of the lod scores obtained. The effect, however, is likely to be small. In the present case this theoretical disadvantage was outweighed by the benefits of reduced computational demands for this extremely complex pedigree. Two-point and multipoint lod scores were calculated with the LINKAGE package of programs (Lathrop et al 1984) for the polymorphic markers listed in Table 1.

Three three-point analyses were performed between affection status, HRAS and TH to examine D4 (Gelernter et al 1992); affection status, DRD2 and D11S97 to exclude tyrosinase (Barton et al 1988) and between affection status, DRD1 and D5S211 to examine the role in susceptibility for the D1 gene (Wasmuth et al 1991).

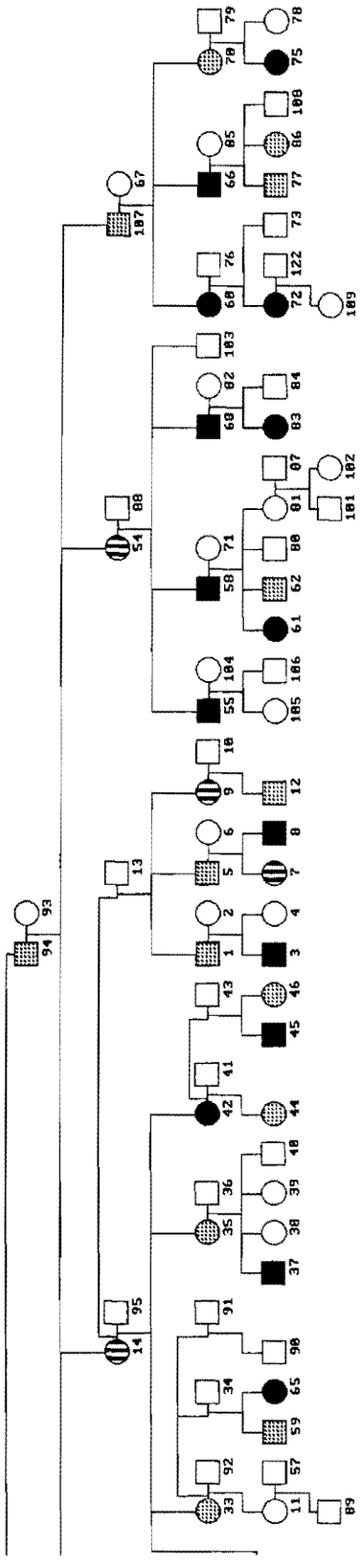

Figure 1 continued

Table 1. Linkage Markers, Their Location and Allele Frequencies Used in the Study

Probe name	Locus	Chromosomal location	References
c-Ha-RAS1	HRAS	11p15.5	(Gelernter et al 1992)
THCA	TH	11p15.5	(Gelernter et al 1992)
pMS51	D11S97	11q13	(Barton et al 1988)
D2-5/D2-6	DRD2	11q22-q23	(Barton et al 1988)
DBH-CA	DBH	9q34.3	(Porter et al 1992)
HGR213-1	DRD1	5q34-35	(Wasmuth et al 1991)
Mfd154CA	D5S211	5q33.3-qter	(Wasmuth et al 1991)
D5CA	DRD5	4p16	(Sherrington et al 1993)
L214	DRD3	3q13.3	(Lannfelt et al 1992)

Probe	Alleles	Frequency
HGR213-1	EcoRI A1	0.90
	A2	0.10
L214	MscI A1	0.72
	A2	0.28
D2-5/D2-6	PCR A1	0.15
	A2	0.47
	A3	0.22
	A4	0.16

All the other markers were condensed to five alleles with equal fequencies for the lod score calculations.

Results

The two-point lod scores for each marker used are shown in Table 2. The genetic information from the probe for the D1 receptor gene was largely uninformative, but by using a tightly linked microsatellite at D5S211 we were able to exclude the region around the DRD1 locus over a distance of 20 centimorgans with a two-point analysis and over 40 centimorgans using a three-point lod analysis (Figure 2). The gene for the enzyme tyrosinase is localized on chromosome 11q between the DRD2 and D11S97 loci and is excluded using a three-point analysis, (Figure 3). A three-point analysis using markers at the HRAS and tyrosine hydroxylase loci was used to exclude the role of the D4 receptor in GTS (Figure 4). All the other loci of interest, DRD2, DRD3, DRD5, DBH, and TH were excluded with two-point analyses using their associated polymorphisms (Table 2).

Discussion

There have been several previous linkage analyses reported in GTS, examining various candidate loci or general exclusions of specific chromosomes. Devor et al (1990) and Gelernter et al (1990) both previously excluded a role for the D2 dopamine receptor in GTS. Comings et al (1993) reported increased homozygosity for the D3 receptor gene, but Brett et al (1993) failed to replicate this finding. Heutink et al (1990) excluded chromosomes 7 and 18, and Pakstis et al (1991) combined the exclusion data from several groups to exclude more than 50% of the genome. Brett et al (1989,

Table 2. Two-Point Lod Scores for Linkage between Affection Status and Nine Markers for Two Diagnostic Models

Theta	0.000	0.001	0.010	0.050	0.100	0.200	0.300	0.400
Two-point lod scores for HRAS								
GTS	−6.946	−6.201	−4.281	−2.541	−1.860	−1.117	−0.574	−0.201
GTS & CMT	−16.199	−14.280	−10.219	−5.835	−3.912	−1.993	−0.815	−0.194
Two-point lod scores for D11S97								
GTS	−9.443	−8.260	−6.232	−4.142	−2.925	−1.523	−0.776	−0.394
GTS & CMT	−21.255	−19.568	−14.169	−7.693	−4.791	−1.984	−0.723	−0.235
Two-point lod scores for TH								
GTS	−10.909	−10.006	−7.289	−4.243	−2.561	−0.967	−0.208	0.074
GTS & CMT	−23.638	−22.435	−16.944	−9.437	−6.036	−2.760	−1.078	−0.239
Two-point lod scores for DRD2								
GTS	−12.201	−11.777	−9.280	−5.529	−3.595	−1.748	−0.889	−0.396
GTS & CMT	−13.600	−13.029	−10.175	−5.576	−3.185	−1.048	−0.255	−0.048
Two-point lod scores for DBH								
GTS	−8.903	−7.726	−5.455	−3.145	−1.946	−0.692	−0.173	−0.013
GTS & CMT	−8.539	−6.614	−3.554	−1.104	−0.241	0.439	0.636	0.445
Two-point lod scores for DRD1								
GTS	0.806	0.804	0.784	0.698	0.591	0.387	0.213	0.083
GTS & CMT	0.016	0.023	0.085	0.271	0.383	0.408	0.312	0.169
Two-point lod scores for D5S211								
GTS	−10.655	−10.443	−8.914	−5.782	−3.951	−1.985	−0.954	−0.358
GTS & CMT	−14.928	−14.087	−11.419	−6.917	−4.246	−1.777	−0.697	−0.202
Two-point lod scores for DRD5								
GTS	−12.858	−11.914	−9.427	−5.769	−3.862	−1.908	−0.784	−0.183
GTS & CMT	−22.939	−20.703	−16.289	−10.531	−7.102	−3.598	−1.610	−0.481
Two-point lod scores for DRD3								
GTS	−3.204	−3.071	−2.516	−1.659	−1.126	−0.567	−0.264	−0.088
GTS & CMT	−6.263	−5.720	−4.195	−2.267	−1.345	−0.601	−0.300	−0.128

1990, 1991) examined in detail the association of a reciprocal translocation, between chromosomes 3p and 8q, found in a rare case of GTS and excluded most of chromosomes 3p and 8q from being involved in the etiology of GTS.

Given the difficulty experienced so far in localizing a susceptibility gene for GTS and related disorders, methods to overcome the possibility that there is heterogeneity of linkage in the genetic predisposition to GTS must be considered. We have chosen the approach used by Gelernter et al (1990, 1993), of primarily studying a single large kindred, in which it may be assumed a single locus is responsible. We routinely follow-up weakly positive lod scores in the main kindred by studying a number of smaller families. Our results suggest that abnormalities in the cloned human dopamine receptors and the catecholamine metabolizing enzymes studied do not have a major effect in the etiology of GTS in the family studied by us, assuming that the mode of transmission is approximately correct, which is that the cases of GTS in this family are due to the action of a single autosomal dominant gene with fairly high penetrance. It does of course remain a possibility that abnormalities of these genes may cause GTS in other families.

In excluding these genes we have only investigated a small number of the potential candidate genes of neurological relevance that have recently been cloned. The involvement of serotonergic system genes has been proposed (Crosley 1979). In addition there are many second messenger systems that might be involved in the etiology of GTS (Singer and Walkup 1991). Genes encoding proteins involved in the gammaaminobutyric acid (GABA)ergic, serotonergic, adrenergic, and cholinergic systems should also be studied using linkage methods.

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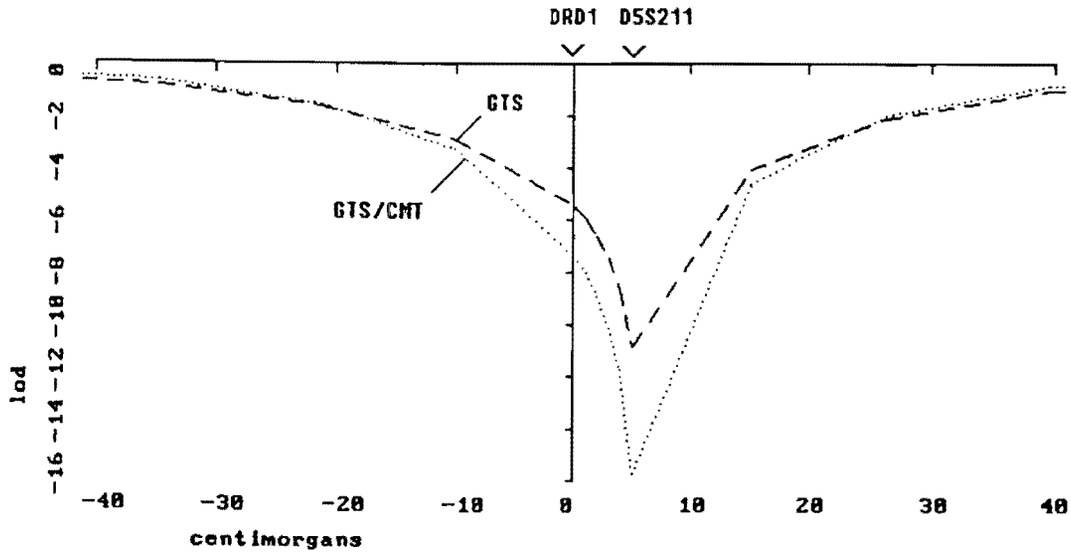

Figure 2. Multipoint lod scores on chromosome 5 between GTS/CMT and D5S211 and DRD1 to exclude the dopamine D1 receptor gene. DRD1 is at 0cM and D5S211 is at 5cM.

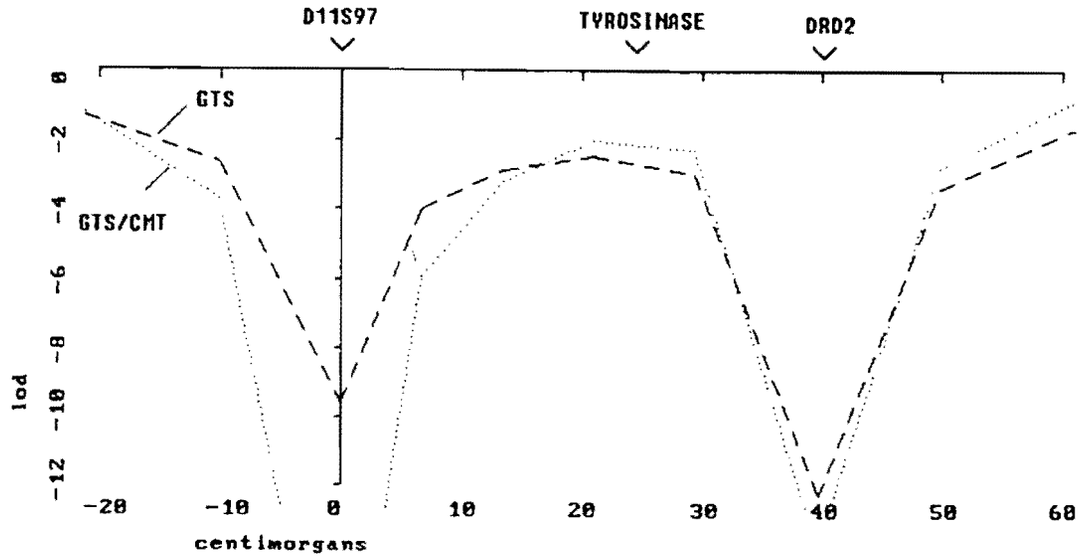

Figure 3. Multipoint lod scores on chromosome 11 between GTS/CMT and D11S97 and DRD2 to exclude the gene for tyrosinase. D11S97 is at 0cM and DRD2 is at 40 cM, with the tyrosinase gene lying at 25 cM from D11S97 and 15cM from DRD2.

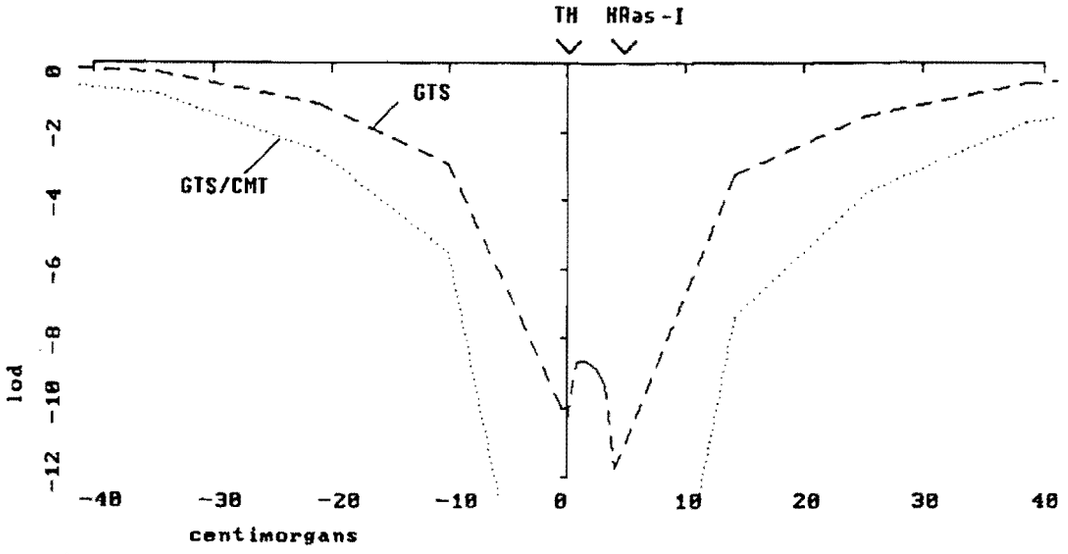

Figure 4. Multipoint lod scores on chromosome 11 between GTS/CMT and TH and HRAS to exclude the gene for D4 dopamine receptor. TH is at 0cM and HRAS-1 is at 3.8cM, with the DRD4 gene lying 2cM. either proximal or distal, of HRAS-1.

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Linkage analysis and exclusion of regions of chromosomes 3 and 8 in Gilles de la Tourette syndrome following the identification of a balanced reciprocal translocation 46 XY, t(3:8)(p21.3 q24.1) in a case of Tourette syndrome

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Gilles de la Tourette syndrome (GTS) and related disorders such as chronic multiple tics and obsessive compulsive behaviour are likely to be genetically transmitted with a Mendelian autosomal dominant mode of transmission. Following our discovery of a patient with GTS who also carried a balanced translocation 46 XY, t(3:8) (p21.3 q24.1), a linkage study of several families was performed covering the areas on chromosomes 3 and 8 implicated by the cytogenetic abnormality in this unique GTS patient. A positive multipoint lod score of 2.9 was obtained on chromosome 3 with markers at the loci RAF1, THRB, and D3S11. Subsequently, the genetic map of this region was improved and new polymorphic markers close to our original three markers were identified. With the new map the maximum two-point lod with any marker was reduced to 1.77 at RAF1, and the FASTMAP approximate multipoint lod excluded the likely region of the breakpoint. After constructung a somatic cell hybrid, the original three markers were mapped relative to the break point of the translocation and to other new markers. It was confirmed that the original markers were at least 20 cM away from the position of the break point. In addition, we traced further family members of our translocation GTS proband, and identified affected individuals who did not possess the translocation. We concluded that the translocation was not responsible for the GTS symptoms in our affected proband.

Keywords: Linkage analysis – Chromosome 3 – Chromosome 8 – Gilles de la Tourette Syndrome – Translocation 46 XY t(3:8) (p21.3 q24.1) – Cytogenetic abnormality

INTRODUCTION

We report on a linkage study of a large family multiply affected with Gilles de la Tourette syndrome (GTS), chronic multiple tics (CMT) and obsessive compulsive behaviour (OCB) covering areas on chromosomes 3 and 8 following our discovery of a patient with GTS who also carried a balanced translocation 46 XY, t(3:8) (p21.3 q24.1). GTS is thought to be a genetically determined disease which sometimes has a dominant mode of inheritance with incomplete penetrance and expression as shown by segregation analyses carried out on several independent GTS samples (Devor, 1984; Pauls and Leckmann, 1986; Curtis *et al.*, 1992; Eapen *et al.*, 1993). Because of the hereditary nature of GTS and the discovery of the translocation, markers on chromosome 3 and chromosome 8 were used on an unrelated large British kindred multiply affected with GTS, CMT and OCB (Robertson and Gourdie, 1990) to test the hypothesis that there would be linkage at the loci implicated by the cytogenetic abnormality. Three markers on chromosome 3 (THRB, E41 and RAF1) produced positive lod scores (Brett *et al.*, 1990), so the area surrounding these markers was examined by using a further 16 linked markers. Cells were fused using the PEG method as described by Carritt and Povey (1979). A cell hybrid which contained the abnormal chromosome 8 was found and used to map the break points relative to the markers used. Chromosome 8 was investigated using 19 markers covering part of the p-arm and all

of the q-arm including the area around the breakpoint.

METHODS

Family collection and diagnosis

The pedigree (F24) used for the linkage analysis was identified when the proband presented at a GTS clinic at the National Hospital for Neurology and Neurosurgery, Queen Square. The pedigree studied consists of 116 members, 85 of whom were individually interviewed. The structure and paternity were confirmed using DNA fingerprinting.

One of us (M.M.R.) evaluated individuals in the pedigree. Subjects were interviewed to assess 'caseness' using a semi-structured diagnostic interview to obtain DSM III criteria for GTS and CMT which has been developed by M.M.R. (Robertson *et al.*, 1988). The protocol used is highly comparable with that used by other workers in the field. Other psychiatric diagnoses were made using the SADS-L standardized interview (Spitzer *et al.*, 1977) and Research Diagnostic Criteria (RDC) (Spitzer *et al.*, 1978).

Patients were assigned to diagnostic categories as follows:

(1) Definite or probable GTS; satisfying DSM III (APA, 1980) criteria on history and/or examination.
(2) Definite or probable CMT; DSM III diagnosis on history and/or examination.
(3) Obsessive compulsive behaviours (OCB) without GTS or CMT; obtained on history and, in the case of children, corroborated by parents.

Of the 85 interviewed 29 had GTS, a further 20 had CMT without GTS and five had OCB alone (Robertson and Gourdie, 1990).

RFLP and microsatellite polymorphism determination

DNA was isolated from peripheral lymphocytes (Sambrook *et al.*, 1989). Five to 10 μg DNA was digested with 20–30 units of restriction enzymes overnight in buffers recommended by the manufacturer (Boehringer Mannheim or Amersham). The DNA was electrophoretically separated on 0.8% agarose gels and transferred to a nylon membrane (Hybond^N, Amersham) according to the manufacturers' protocols. The DNA was fixed to the membrane by UV irradiation. Prior to hybridization the blots were prehybridized in 0.9 M NaCl, 1% SDS with sheared salmon sperm DNA at a final concentration of 50 mg ml^{-1} for at least 4 h. Plasmid DNA from the probes was isolated according to the alkaline lysate procedure of Birnboim and Doly (1979). Insert DNA was prepared by electroelution or by separation in low-melting agarose (Sambrook *et al.*, 1989). The insert DNA was labelled by random priming (Feinberg and Vogelstein, 1983) with ^{32}P-dCTP (Amersham) to a specific activity of $2–10 \times 10^8$ cpm μg^{-1} and, after denaturation, added to the hybridization buffer (0.9 M NaCl, 1% SDS, 10% dextran sulfate, 50 mg ml^{-1} sheared salmon sperm DNA). Hybridization was at 65°C overnight. The blots were washed once in 1 × SSC, 0.1% SDS for 20 min at 65°C, followed by two washes at 65°C in 0.5–0.1 SSC, 0.1% SDS for 20 min each. The hybridized filters were autoradiographed for up to 14 days using Fuji X-ray film at −70°C.

PCR was performed in 12.5 μl containing 50 ng DNA, 12.5 pmol of each primer, 1.0 mM $MgCl_2$, 10 mM Tris-Cl pH 8.3, 50 mM KCl, 25 μM dATP and 200 μM of other dNTPs, 1 unit Taq polymerase (Perkin-Elmer/Cetus), 0.01% gelatin and 0.5 μl. End labelled primers were used for the microsatellite amplifications (100 pmol of primer labelled in 10 μl with 1 μl gamma-^{32}P ATP at 3000 Ci mmol^{-1}, 0.1 μl of this was used per reaction). Amplification was for 35 cycles with denaturation at 94°C, annealing at the appropriate temperature for the primers and 20 s extension at 72°C. The amplified product was separated by polyacrylamide gel electrophoresis using a urea denaturing gel. The gels were fixed in 10% acetic acid/10% methanol and vacuum dried on to 3 mm paper. The gels were autoradiographed using Fuji X-ray film at −70°C for 1 h to 2 days depending on the primers used.

Genetic analysis

Two diagnostic categories were used to indicate positive affection status, GTS only and GTS and CMT. Based on the results of a previous segregation analysis (Curtis *et al.*, 1992), autosomal transmission was assumed with a gene frequency of 0.0005, and heterozygote penetrances of 0.5 for GTS and 0.88 for GTS and CMT. To allow for occasional phenocopies, the normal homozygote penetrance was set to 0.001. No separate analysis was carried out including the OCB cases as affected, because four out of five of the OCB cases were obligate carriers under the assumption of dominant transmission. Two-point lod scores were calculated with FASTLINK (Cottingham *et al.*, 1993; Schaffer *et al.*, 1994) for the polymorphic markers listed in Tables I and II. Markers used and their allele frequencies are shown in Tables I and

TABLE I. Two-point lod scores for linkage between the two models of affection status and the markers used on chromosome 3

Theta	0.000	0.010	0.050	0.100	0.200	0.300
THRB microsatellite and RFLP						
GTS	−2.095	−1.059	−0.498	−0.325	−0.218	−0.146
GTS and CMT	−4.669	−2.315	−1.063	−0.553	−0.136	0.018
RAF						
GTS	1.768	1.907	2.338	2.365	1.865	1.131
GTS and CMT	−3.291	−1.363	0.265	1.107	1.398	0.998
R59						
GTS	−2.673	−2.560	−1.914	−1.338	−0.705	−0.354
GTS and CMT	−5.269	−4.187	−2.697	−1.788	−0.938	−0.495
D3F15S2E						
GTS	0.246	0.493	0.784	0.821	0.661	0.423
GTS and CMT	−3.831	−2.164	−0.850	−0.306	0.072	0.139
D3S196						
GTS	−10.351	−7.958	−5.013	−3.429	−1.759	−0.827
GTS and CMT	−16.025	−10.438	−5.995	−4.072	−2.203	−1.192
D3S17						
GTS	−8.784	−5.351	−2.702	−1.515	−0.372	0.154
GTS and CMT	−14.237	−8.151	−4.337	−2.494	−0.688	0.013
D3S11						
GTS	0.399	0.667	1.012	1.100	0.997	0.733
GTS and CMT	0.591	1.534	2.020	2.065	1.785	1.298
D3S5						
GTS	1.344	1.319	1.212	1.069	0.766	0.461
GTS and CMT	−1.219	−0.075	0.539	0.719	0.690	0.468
CP						
GTS	−4.020	−3.186	−2.241	−1.605	0.755	−0.300
GTS and CMT	−5.998	−4.003	−1.967	−0.851	−0.056	0.078
D3S18						
GTS	−2.554	−1.596	−0.648	−0.294	−0.080	−0.027
GTS and CMT	−5.199	−3.867	−2.172	−1.219	−0.387	−0.061
D3S1312						
GTS	−7.768	−6.019	−3.743	−2.601	−1.351	−0.638
GTS and CMT	−12.280	−7.365	−3.779	−2.053	−0.486	−0.010
D3S1284						
GTS	−6.467	−4.059	−1.773	−0.755	−0.066	0.033
GTS and CMT	−11.685	−7.339	−3.553	−1.543	0.031	0.422
D3S1281						
GTS	−6.421	−4.894	−2.531	−1.206	−0.172	0.124
GTS and CMT	−16.675	−10.200	−5.473	−3.062	−1.003	−0.216
LIB12-37						
GTS	−7.758	−6.189	−3.726	−2.386	−1.122	−0.504
GTS and CMT	−9.191	−6.791	−4.100	−2.707	−1.216	−0.508
D3S1100						
GTS	−9.217	−6.734	−3.894	−2.557	−1.155	−0.435
GTS and CMT	−17.725	−10.912	−5.796	−3.679	−1.675	−0.627

TABLE II. Two-point lod scores for linkage between affection status and the markers used on chromosome 8

Theta	0.000	0.010	0.050	0.100	0.200	0.300
TG						
GTS	−0.881	−0.879	−0.833	−0.622	−0.171	0.008
GTS and CMT	−1.819	−1.095	−0.451	−0.133	0.179	0.206
CA2						
GTS	−2.838	−2.207	−1.570	−1.170	−0.616	−0.282
GTS and CMT	−1.261	−0.514	0.061	0.277	0.411	0.378
CA3						
GTS	−3.852	−3.199	−2.144	−1.441	−0.734	−0.371
GTS and CMT	−4.333	−4.061	−2.880	−2.012	−1.082	−0.570
NEFL						
GTS	−2.070	−1.872	−0.989	−0.453	0.011	0.153
GTS and CMT	−2.371	−1.692	−1.053	−0.559	0.080	0.283
PLAT						
GTS	0.333	0.324	0.285	0.233	0.129	0.048
GTS and CMT	1.375	1.339	1.197	1.020	0.689	0.408
D8S39						
GTS	−10.130	−7.724	−4.653	−2.935	−1.260	−0.484
GTS and CMT	−9.577	−6.776	−3.453	−1.644	−0.130	0.353
D8S38						
GMT	−0.906	0.027	0.875	1.183	1.253	0.962
GMT and CMT	−2.935	−1.809	−0.244	0.380	0.748	0.654
D8S11						
GTS	−0.880	−0.347	0.163	0.344	0.414	0.343
GTS and CMT	−1.119	−0.562	−0.017	0.192	0.301	0.266
D8S7						
GTS	0.663	0.665	0.656	0.620	0.512	0.385
GTS and CMT	−0.383	−0.371	−0.307	−0.166	0.087	0.156
D8S8						
GTS	−0.377	0.351	0.809	0.886	0.765	0.541
GTS and CMT	−4.797	−2.670	−0.932	−0.253	0.216	0.294
PENK						
GTS	−1.877	−0.862	0.075	0.384	0.474	0.360
GTS and CMT	−4.536	−2.901	−1.069	−0.274	0.299	0.383
D8S556						
GTS	−5.411	−4.079	−2.550	−1.480	−0.390	0.075
GTS and CMT	−16.521	−10.862	−6.251	−3.854	−1.613	−0.494
D8S284						
GTS	−5.392	−3.770	−1.700	−0.779	0.040	0.316
GTS and CMT	−13.358	−8.015	−3.751	−2.027	−0.474	0.161
D8S554						
GTS	−8.539	−5.649	−2.778	−1.112	0.144	0.405
GTS and CMT	−14.038	−9.446	−5.697	−3.128	−0.662	0.217
D8S272						
GTS	−7.477	−5.140	−2.111	−0.715	0.346	0.563
GTS and CMT	−10.264	−3.617	−0.075	1.212	1.898	1.677
D8S373						
GTS	−10.097	−7.998	−4.636	−2.834	−1.218	−0.510
GTS and CMT	−15.047	−9.430	−4.646	−2.632	−0.941	−2.222

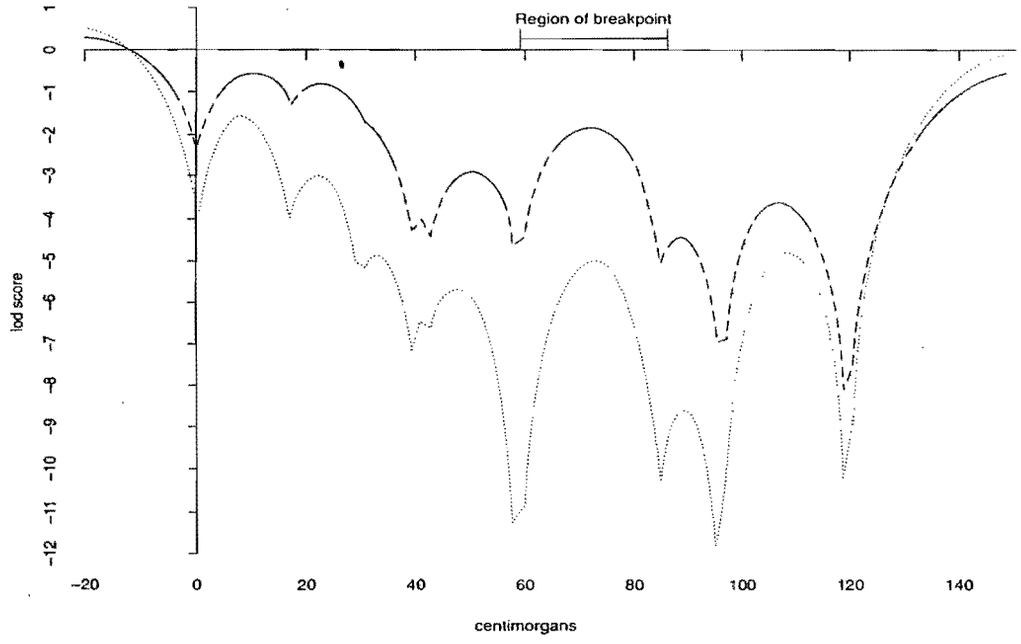

FIG. 1. Approximate multipoint lod scores on chromosome 3 obtained from FASTMAP by combining results from two-point analyses. (Map order and distances from the Genome database and Gyapay *et al.*, 1994.) D3S17 is at 0 cM; D3S18 at 3 cM; RAF1 at 6 cM; THRB (RFLP and microsatellite) at 26 cM; D3S12 at 30 cM; D3F15S2 at 42 cM; D3S1312 at 57 cM; D3S1284 at 77 cM; D3S1281 at 97 cM; and D3S196 at 137 cM. The break point is located between D3S1312 and D3S1100, which is at approximately 32 cM on this map. The dashed line indicates the GTS affection model, while the dotted line includes both GTS and CMT cases as affected.

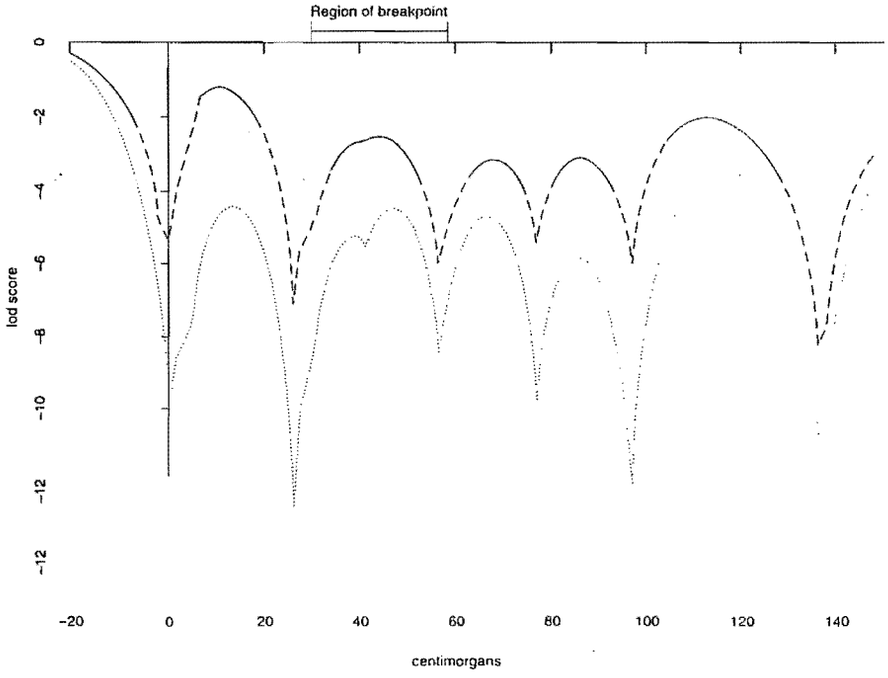

FIG 2. Approximate multipoint lod scores on chromosome 8 obtained from FASTMAP by combining results from two-point analyses. (Map order and distances from the Genome database and Gyapay *et al.*, 1994.) NEFL is at 0 cM; PLAT at 8 cM; PENK at 17 cM; D8S8 at 30 cM; CA3 at 39 cM; CA2 at 43 cM; D8S556 at 59 cM; D8S284 at 85 cM; D8S554 at 97 cM; D8S272 at 98 cM; and D8S373 is at 121 cM. The break point is located between markers D8S556 and D8S284. The dashed line indicates the GTS affection model, while the dotted line includes both GTS and CMT cases as affected.

II. Multiple two-point lod scores were combined into approximate multipoint maps using FASTMAP (Curtis and Gurling, 1993).

RESULTS

Investigation of the GTS family with translocation

The mother was a definite case of GTS and a blood sample was found to be positive for the 3:8 translocation. Of the other family members on the maternal side, about whom information was obtained, nine out of 14 had a diagnosis of GTS. Further blood samples were taken from the maternal grandfather, uncle and aunt, who all had a diagnosis of GTS, to see if the translocation co-segregated with the GTS found in this family. The cytogenetic screens carried out showed that the proband (AB) and his mother were the only carriers of the translocation and therefore no further cytogenetic screens were undertaken on the other maternal relatives. From this we drew the conclusion that the translocation was unlikely to be involved in the actiology of the GTS and related disorders in this family.

Linkage analyses

The markers E41, p627 and pBH302 produced a maximum lod score of 2.998 in an earlier multipoint analysis (Brett *et al.*, 1990) using the map order THRB-D3S11-RAF1. This map order, however, proved to be inaccurate (CHLC, 1993). Following this report many more markers in this region of chromosome 3 were typed and lod scores calculated (Table III). Eleven of the markers were used for a FASTMAP analysis. The position of one of the original three markers (D3S11) was omitted due to lack of certainty concerning the actual distances from flanking markers as was D3S1100, for the same reason. The break point found in AB was mapped relative to the new markers and was found to be flanked by D3S1100 and D3S1312 in a gap of 25 cM, which places the break point at least 20 cM proximal to the positive lod score. With the new markers the region from D3S17 to D3S196 was excluded (lod < -2.00) using the FASTMAP program as shown in Fig. 1.

On chromosome 8 a few small, non-significant positive lod scores (Table IV) were observed due mainly to a lack of heterozygosity in many of the older RFLP markers. These were investigated further by performing a FASTMAP analysis across some of the p-arm and all of the q-arm of chromosome 8 with 11 linked markers. This proved to be negative (lod < -2.00) for the whole map (Fig. 2), and therefore the whole region was excluded from NEFL at 8p21 to D8S373 at the telomere of 8q. The break point on chromosome 8 was found to be in a 26 cM gap flanked by the markers D8S556 and D8S284, both of which were used in the FASTMAP analysis.

DISCUSSION

From the results obtained using improved map data and further markers in the region surrounding the original positive lod score it was concluded that the original lod score did not reflect any involvement of the relevant chromosomes 3 and 8 in the aetiology of GTS in the family studied. The cytogenetic screens carried out showed that the proband and his mother were the only carriers of the translocation. From this the conclusion drawn was that the translocation was not likely to be involved in the aetiology of the GTS and related disorders in this family. Furthermore, from work carried out on the cell hybrids from the proband we found that the actual chromosome 3 break point was localized proximal to the region producing the positive lod score.

The results must, however, be interpreted with the possibility of heterogeneity in mind. This means that these exclusions may not apply to all GTS families being studied by linkage methods. To date approximately 85% of the genome has been covered in the search for the predisposing gene in GTS (Pakstis *et al.*, 1991; USA Tourette Syndrome Association Collaborative Group, personal communication) assuming homogeneity. Considerably less has been excluded assuming heterogeneity.

The approach of examining candidate genes and regions implicated by cytogenetic abnormalities is a potentially fruitful one in the current situation where a confirmed linkage has yet to be found. There have been a few other instances of reported associations between GTS and cytogenetic abnormalities but these have not resulted in the localization of the predisposing gene, for example on chromosome 9p (Singh, 1982; Taylor, 1990). With much of the genome already screened, further randomly chosen markers should soon localize a susceptibility gene for GTS but this is likely to reault from work carried out in a single large kindred.

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Neuroreceptor Subunit Genes and the Genetic Susceptibility to Gilles de la Tourette Syndrome

Peter M. Brett, David Curtis, Mary M. Robertson, and Hugh M.D. Gurling

Segregation studies have shown that Gilles de la Tourette Syndrome (GTS) is probably transmitted as an autosomal dominant gene disorder and can therefore be studied by classical linkage analysis to identify susceptibility loci. Many neurotransmitter systems have been implicated in the etiology of GTS. Most recently the alpha-1 subunit of the glycine receptor etiologically responsible for hyperekplexia has been hypothesized as the cause of the susceptibility to GTS. Because of this and the high concentration of other neuroreceptor genes at 5q33–35, it was decided to study this region and the associated gene cluster on chromosome 4p12–16 in a large British kindred multiply affected with GTS and chronic motor tics. The genotypes of the microsatellite markers at these loci were determined by polymerase chain reaction. The allele data were analyzed using both parametric and nonparametric methods. Approximate multipoint maps were constructed across the regions of interest using FASTLINK. All of the lod scores produced were negative, showing no evidence of linkage to GTS in the family studied. The multipoint maps showed good exclusion across these regions. The glycine receptor gene responsible for hyperekplexia and the other neuroreceptor genes examined in this paper are not involved in the etiology of GTS in this large pedigree. © *1997 Society of Biological Psychiatry*

Key Words: Gilles de la Tourette Syndrome, hyperekplexia, glycine receptor GABA receptors, linkage

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Introduction

Gilles de la Tourette syndrome (GTS), first described in 1825 by Itard and later in 1885 by Georges Gilles de la Tourette (1899), is a neurological disorder characterized by multiple motor and one or more vocal tics (American Psychiatric Association 1980; World Health Organization 1992). When first described GTS was thought to be rare, but the currently accepted lifetime prevalence is of at least 0.5 per thousand (Robertson 1989). Segregation analyses carried out on independent GTS samples have shown GTS to be genetically determined, with an autosomal dominant mode of transmission with incomplete penetrance (Comings et al 1984; Devor 1984; Price et al 1984; Pauls and Leckmann 1986; Curtis et al 1992; Eapen et al 1993). There have been several twin studies that have given concordance data showing pleiotropy for the genetic susceptibility for GTS (Shapiro and Shapiro 1980; Jenkins et al 1983; Waserman et al 1983; Price et al 1984).

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Hereditary startle disease or hyperekplexia (STHE) is an autosomal dominant neurologic disorder that is caused by a base change in exon 6 of the alpha-1 subunit of the glycine receptor (Shiang et al 1993). As such it is the first neurologic disorder found to be caused by a mutation in a neuroreceptor gene. It has been hypothesized that GTS may be caused by a mutation in the same gene, since GTS has exaggerated startle responses as a part of the symptomatology (Floeter and Hallett 1993). The gene encoding for the glycine alpha1 subunit (GLRA1) is located on chromosome 5q in an area rich with genes encoding for neuroreceptors. This region contains the genes for: gamma-aminobutyric acid $(GABA)_A$ receptor alpha-1, alpha-6, and gamma-2 subunits (GABRA1, GABRA6, and GABRG2); dopamine D1 receptor; glutamate receptor GLUR1; the alpha adrenergic receptor ADRA1B; the beta adrenergic receptor ADRB2, and the glucocorticoid receptor GRL (Hicks et al 1994; Shiang et al 1993; Warrington et al 1991, 1992; Ryan et al 1992; Johnson et al 1992).

Due to this high concentration of neuroreceptor genes, it was decided to perform a linkage study of the whole region in a large British kindred multiply affected with GTS and chronic motor tics (CMT). We have chosen to study this large kindred as it may be assumed that a single locus is responsible for the susceptibility to GTS. CMT and obsessive–compulsive behaviors are thought to be variant phenotypes of the GTS susceptibility gene (Kurlan 1989; Eapen et al 1993). The $GABA_A$ receptor beta-1 and alpha-2 subunits (GABARB1 and GABARA2), which lie within 6 cM of each other on chromosome 4p (Dean et al 1991; Wilcox et al 1992; Greger et al 1995; Genome Database of Johns Hopkins University), and the gene encoding for dopamine transporter (DAT1) on chromosome 5p (Vandenbergh et al 1992) have been suggested to be involved in the etiology of GTS (Caine 1985; Cook et al 1995) and are, therefore, also good candidates for study by linkage analysis. The linkage study was performed using microsatellite markers linked to the genes that were hypothesized to have a role in the susceptibility to GTS.

Methods

Family Collection and Diagnosis

The pedigree was identified when the proband presented at a GTS clinic at the National Hospital for Neurology and Neurosurgery, Queen Square. The pedigree studied consists of 116 members, 85 of whom were individually interviewed. The structure and paternity of the kindred were confirmed using DNA fingerprinting.

One of us (MMR) evaluated individuals in the pedigree. Subjects were interviewed to assess "caseness" using a semistructured diagnostic interview to obtain DSM-III criteria for GTS and chronic multiple tic syndrome (CMT) that has been developed by MMR and used regularly at the National Hospital Queen Square (Robertson et al 1988). The protocol used is highly comparable with other workers in the field. Other psychiatric diagnoses were made using the Schedule for Affective Disorders and Schizophrenia–Lifetime (SADS-L) standardized interview (Spitzer and Endicott 1977) and research diagnostic criteria (RDC) (Spitzer et al 1978).

Patients were assigned to diagnostic categories as follows:

1. Definite or probable GTS; satisfying DSM-III (American Psychiatric Association 1980) criteria on history and/or examination.
2. Definite or probable CMT; DSM-III diagnosis on history and/or examination.
3. Obsessive–compulsive (OC) behaviors without GTS or CMT; SADS-L and RDC diagnoses obtained on history and, in the case of children, corroborated by parents.

Of the 85 interviewed, 35 had GTS, a further 14 had CMT without GTS, and 5 had OC behaviors alone (Robertson and Gourdie 1990).

Microsatellite Polymorphism Determination

Polymerase chain reaction (PCR) was performed in 12.5 μL containing: 50 ng DNA, 12.5 pmol of each primer, 1.0 mmol/L $MgCl_2$ 10 mmol/L Tris-Cl pH 8.3, 50 mmol/L KCl, 200 μmol/L of 2′-deoxynucleoside 5′-triphosphate, 1 unit Taq polymerase (Perkin-Elmer/Cetus), 0.01% gelatin. End-labeled primers were used for visualization of microsatellite amplification products (100 pmol of primer labeled in 10 μL with 1 μL gamma-^{32}P adenosine triphosphate at 3000 Ci/mmol; 0.1 μL of this was used per reaction). Amplification was for 35 cycles with denaturation at 94°C, annealing at the appropriate temperature for the primers and 20 sec extension at 72°C. The amplified product was separated by polyacrylamide gel electrophoresis using a urea denaturing gel. The gels were not fixed or vacuum dried, and were autoradiographed using Fuji X-ray film at −70°C for 1 hour to 2 days, depending on the primers used.

Linkage Analysis

Two diagnostic categories were used to indicate positive affection status, GTS only and GTS and CMT. Based on the results of a previous segregation analysis (Curtis et al 1992), autosomal transmission was assumed with a gene frequency of .0005, and heterozygote penetrances of .5 for GTS and .88 for GTS and CMT. To allow for occasional

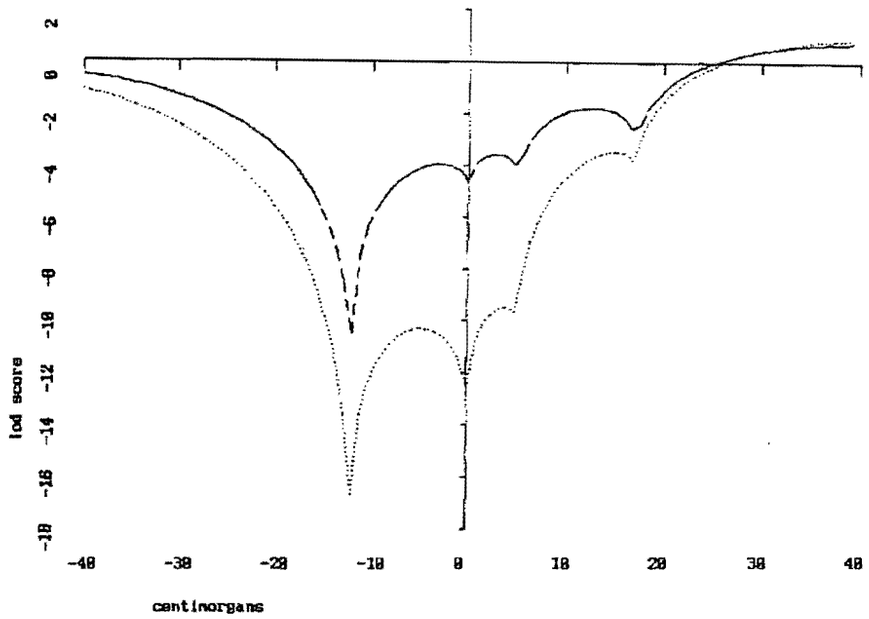

Figure 2. Approximate multipoint lod scores obtained from FASTMAP by combining results from two-point analyses, with D4S174 at −12 cM, GABRB1 at 0 cM, D4S398 at 5 cM, and D4S1558 at 17 cM. GABRA2 maps between D4S174 and GABRB1 at 6 cM from D4S174. The dashed line indicates the GTS affection model; the dotted line includes both GTS and CMT cases as affected.

match the patterns of inheritance found in GTS pedigrees, and that this may account for the lack of positive linkage in GTS. To exclude the possibility of model misspecification, we also used nonparametric analyses, which make no assumption about the mode of transmission and confirmed our findings.

The chromosomal regions excluded in the present study contain the genes GABRA1, GABRA6, GABRG2, GABRB1, GABRA2, GRL, GLUR1, GLRA1, ADRA1, ADRB2, and DRD1. These have all been mapped to these regions by radiation hybrid mapping and/or linkage analysis (Hicks et al 1994; Shiang et al 1993; Warrington et al 1991, 1992; Ryan et al 1992; Johnson et al 1992). The exclusion of these genes has added to the number of potential candidate genes of neurological relevance that have recently been investigated. It does of course remain a possibility that if there is locus heterogeneity present in GTS, abnormalities of these genes may cause GTS in other families. There are, however, many other possible candidate genes still to be examined. In addition to the main neuroreceptor groups hypothesized to be involved, there are many second messenger systems that might be involved in the etiology of GTS (Singer and Walkup 1991; Singer et al 1995). The strategy of examining candidate genes may yet yield the identity of some susceptibility genes for GTS.

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Genome scan of Tourette syndrome in a single large pedigree shows some support for linkage to regions of chromosomes 5, 10 and 13

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Objectives To localize genes influencing the susceptibility to Gilles de la Tourette syndrome (GTS) and associated chronic multiple tics (CMT).

Method A single, large, multiple affected pedigree containing 35 subjects diagnosed with GTS and a further 14 with CMT was genotyped for markers spanning the autosomes. Linkage analysis was carried out using classical lod score analysis and model-free lod score analysis. All markers were subjected to two-point analysis, and markers producing a two-point result significant at $P<0.005$ were subjected to three-point analysis using adjacent markers.

Results The following markers produced at least one result significant at 0.005 using two-point analysis: D5S1981, D5S2050, D10S591, D10S189, D13S217, and D14S288. Three-point analysis with D5S2050 and D5S400 produced a lod of 2.9 with CMT. Three-point analysis of D10S591 and D10S189 produced lods of 1.9 with GTS and CMT. Three-point analysis of D13S217 and D13S171 produced a lod of 2.7 with GTS. No single haplotype appeared to account for the majority of cases within the pedigree.

Conclusions It seems likely that more than one susceptibility allele is present in the pedigree. Although none of the three positive regions is conclusively implicated, it seems probable that at least one contains a susceptibility locus. We recommend that association-based studies be carried out in these three regions to produce further evidence for a localization and to carry out fine-mapping. *Psychiatr Genet* 14:83–87 © 2004 Lippincott Williams & Wilkins.

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Keywords: Tourette, linkage, genome scan

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Introduction

Although there is good evidence that the familial aggregation of Tourette syndrome is at least in part due to the effects of single major loci (Pauls and Leckman, 1986; Curtis *et al.*, 1992; Hasstedt *et al.*, 1995; Walkup *et al.*, 1996), attempts to date to localize the genes concerned have not been definitively successful. A number of linkage and association studies that were claimed to be positive have not been convincingly replicated. A genome screen of 76 families containing 110 affected sib-pairs produced multipoint maximum-likelihood scores (MLSs) greater than 2 at 4q and 8p, and MLSs greater than 1 in four other regions (Tourette Syndrome Association International Consortium for Genetics, 1999). Another genome scan in multigenerational families did not produce any notable evidence for linkage using lod score analyses (Barr *et al.*, 1999). Some positive results were obtained using the affected pedigree method, but the authors cautioned that this approach was prone to produce false positives. Perhaps the most promising finding is that a linkage study of a single large French Canadian pedigree that focused on a number of regions previously claimed to demonstrate association did in fact produce support for linkage to markers at 11q23 (Merette *et al.*, 2000). The implicated region included the marker D11S1277, which had demonstrated association in a study of an Afrikaner population (Simonic *et al.*, 1998). Several other loci have been claimed to demonstrate association in single studies, including a number of so-called 'candidate genes', but such results have not been replicated in independent samples.

Overall, although Tourette syndrome appears to have a major genetic contribution to aetiology that in at least

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some pedigrees appears to be due to single gene effects, attempts to map susceptibility genes have to date proved disappointingly unsuccessful. It is clear that at very least locus heterogeneity must exist, with more than one gene contributing to susceptibility. It may also be that the mode of inheritance is not as straightforward as initially hoped and, for example, that oligogenic effects characterized by the combined action of two or more loci might be important.

We report here on the linkage analysis of a large British pedigree containing multiple cases of Tourette syndrome and related disorders using markers covering the autosomal genome. Previously we have used this pedigree to carry out linkage analyses of particular genetic regions (Brett *et al.*, 1991, 1993, 1995a, 1995b, 1996) but this is the first account of a complete genome scan. The previous studies produced negative results for regions of chromosomes 3 and 8, and around genes coding for neuroreceptors and proteins involved in neurotransmitter metabolism.

Methods

Family collection and diagnosis

The pedigree was identified when the proband presented at a Tourette syndrome clinic at the national Hospital for Neurology and Neurosurgery, Queen Square. The pedigree studied consists of 116 members, 85 of whom were individually interviewed. The structure and paternity of the pedigree were confirmed using DNA fingerprinting.

One of us (M.M.R.) evaluated individuals in the pedigree. Subjects were interviewed to assess 'caseness' using a semi-structured diagnostic interview to obtain DSM-III criteria (American Psychiatric Association, 1980) for Gilles de la Tourette syndrome (GTS) and chronic multiple tic syndrome (CMT) that has been developed and used regularly at the National Hospital Queen Square (Robertson *et al.*, 1988). Other psychiatric diagnoses were made using the Schedule for Affective Disorders and Schizophrenia-Lifetime standardized interview (Spitzer and Endicott, 1977) and research diagnostic criteria (Spitzer *et al.*, 1978).

Patients were assigned to diagnostic categories and were counted as affected if they had definite or probable GTS or CMT on history and/or examination according to DSM-III criteria. Of the 85 interviewed, 35 had GTS and a further 14 had CMT (Robertson and Gourdie, 1990). The pedigree extends over six generations, contains two inbreeding loops and has been presented previously in a report which showed that the segregation of GTS and CMT was consistent with the action of a single autosomal dominant gene with incomplete penetrance (Curtis *et al.*, 1992). The pattern of segregation is consistent with one mutation derived from a single founder segregating throughout the entire pedigree, but we cannot exclude the possibility that susceptibility genes might enter the pedigree from more than one source. For the purposes of linkage analysis, two hierarchical affection definitions were used. In the first, denoted GTS, only subjects with GTS were classed as affected while all other subjects were classed as unaffected. In the second, denoted CMT, subjects with GTS or CMT were classed as affected and others as unaffected.

Genotyping

DNA was isolated from whole frozen blood.. Subjects were typed using 393 microsatellite polymorphic markers spanning the genome from the ABI LMS2 (MD10) mapping set. The average distance between these markers is 10 cM. Polymerase chain reaction (PCR) reactions were carried out for each marker individually in a 5 µl reaction volume, containing approximately 50 ng DNA, 2.5 mM Tris–HCl (pH 8.3), 50 mM KCl, 250 µM dNTPs, 0.625 pmol each primer and 0.25 U Ampli *taq* Gold (Applied Biosystems, Foster City, California, USA). Reactions were carried out on a Applied Biosystems 9600 thermal cycler or using an ABI 877 integrated thermal cycler robot. A standard thermocycling profile was used for all markers, and consisted of an initial denaturation of 12 min at 95°C, followed by 10 cycles with denaturation at 95°C for 15 s, annealing at 55°C for 15 s and synthesis at 72°C for 30 s. This was followed by 20 cycles with denaturation at 89°C for 15 s, annealing at 55°C for 15 s and synthesis at 72°C for 30 s, finishing with an extension step of 72°C for 10 min. PCR products for selected sets of markers were pooled, ethanol precipitated, and size-fractionated on a 5% denaturing polyacrylamide gel (Amresco, Ohio, USA) by electrophoresis on an ABI 377XL sequencer. PCR products were sized using the GeneScan® version 2.1 programme, and scored using the Genotyper® version 2.0 programme. Tests for Mendelian inheritance of marker data were carried out and inconsistent genotypes were repeated or omitted.

Linkage analysis

Linkage analysis was carried out using standard lod score methods and using 'model-free' likelihood-based analysis. For lod score analyses, the FASTLINK program was used (Cottingham *et al.*, 1993; Schaffer, 1996). Separate analyses were performed for the GTS and CMT models with penetrances of 0.5 and 0.8 and phenocopy risks of 0.01 and 0.05, respectively. A dominant mode of transmission was assumed, with susceptibility allele frequency of 0.0005.

The 'model-free' analyses were carried out using the MFLINK program (Curtis and Sham, 1995; Curtis *et al.*, 1999) and the accompanying MFMAP utility. MFLINK calculates the likelihood of the data with the disease locus at a given map position using a range of different

dominant and recessive transmission models having different penetrance values and phenocopy probabilities, but all yielding the same disease prevalence (K_p) and parameterized using a single variable, the heterozygote penetrance (f_1, which is varied from 0 to 1). The MLOD is the maximum lod score obtained for any of these transmission models (maximized over f_1). The MFLOD is the difference between the log-likelihood maximized over both f_1 and alpha, the proportion of linked families, *and the* log-likelihood maximized over f_1 but with alpha constrained to 0. Since in the present analysis there is only one pedigree, the likelihoods are maximized with alpha set either to 1 or to 0; that is, under the assumptions that the pedigree does or does not harbour a susceptibility locus at the test position. MFLINK analyses were carried out using both the GTS and CMT affection models, with the population prevalence being set to 0.01 or 0.05, respectively, to match the prevalences used for the lod score analyses. Two-point analyses were carried out with each marker using a test position at a recombination fraction of 0.05 with the marker, and when three-point analyses were carried out the position midway between a pair of adjacent markers was tested.

An initial screen of all the markers using two-point analyses was performed. Each marker yielded two lod scores and four lod scores from the MFLINK analyses (MLOD and MFLOD for the GTS and CMT). Each type of lod score was converted to a likelihood ratio statistic by multiplying by $2\ln(10) = 4.6$. The statistic derived from the conventional lod score was taken to be distributed as a 50:50 mixture of X_1^2 and X_0^2. As originally described (Curtis and Sham, 1995), the likelihood ratio statistic from the MFLOD was taken to be distributed as a 50:50 mixture of X_1^2 and X_0^2. However, since there is only one pedigree this interpretation is likely to be somewhat conservative because MFLOD is not maximized over a range of different values of alpha, and alpha can only take values of 1 or 0. Subsequently (Curtis *et al.*, 1999), it has been shown that $2\ln(10)^*$MLOD can be taken to be distributed as X_1^2. Using these distributions allows P values to be derived so that the different types of lod score can be compared more easily. All regions that yielded a result significant at $P < 0.005$ using any of the methods of analysis were selected for further study.

Additional analyses from regions highlighted by the screening analyses consisted of computing three-point linkage analyses using pairs of adjacent markers. Because of the size of the pedigree and the fact that it included two loops, it proved impossible to carry out these analyses before first downcoding the genotype data so that each marker was recoded as having only five alleles. This was accomplished automatically using the DOLINK program (Cook *et al.*, 1993), which carries out the recoding in such a way as to minimize loss of linkage information. Even when this had been done, it was not possible to incorporate more than two markers at a time in multipoint analysis. Regardless of which method of analysis had produced the significant results, the three-point analyses were carried out using both GTS and CMT models, and obtaining conventional lod scores as well as MLOD and MFLOD statistics.

Results

The following markers produced at least one result significant at 0.005 using two-point analysis: D5S1981, D5S2050, D10S591, D10S189, D13S217, and D14S288. Results for these markers, along with nearby markers showing some support for linkage, are detailed in Table 1, where distances are shown according to those given in the ABI Prism V2 sex-averaged maps (http://gai.nci.nih.gov/

Table 1 Markers showing support for linkage with Gilles de la Tourette syndrome (GTS) and/or associated chronic multiple tics (CMT) using two-point analysis

Map	Name	Lod		MLOD		MFLOD	
		GTS	CMT	GTS	CMT	GTS	CMT
0.0	D5S1981	1.785**	0.023	1.538*	0.210	1.538**	0.000
173.3	D5S2050	0.692†	1.083†	1.099†	1.781**	1.010†	1.781**
177.2	D5S400	0.465	1.008†	0.301	1.482*	0.000	1.396*
13.0	D10S591	1.425*	0.998†	1.738**	0.883†	1.321*	0.442
18.3	D10S189	0.729	1.130†	1.619*	2.399**	1.214*	2.249**
16.0	D13S217	1.579**	0.209	1.319†	0.000	1.225*	0.000
25.0	D13S171	0.084	0.070	0.679	0.094	0.000	0.000
33.1	D13S218	0.744†	1.073†	1.704*	0.707	0.585	0.171
33.3	D14S70	0.533	0.893†	0.732	1.459*	0.000	0.000
40.2	D14S288	1.528**	1.014†	0.660	0.476	0.000	0.000

MLOD, maximum lod score obtained for any transmission model (maximized over f_1); MFLOD, difference between the log-likelihood maximized over both f_1 and alpha, the proportion of linked families, and the log-likelihood maximized over f_1 but with alpha constrained to 0.
*$P<0.01$;
**$P<0.005$;
†$P<0.05$.

ABI/index.html). The full set of results can be inspected online (http://www.mds.qmul.ac.uk/statgen/dcurtis/gtsscan.html). These markers were incorporated in three-point analyses with flanking markers.

The results from three-point analysis provided increased support for linkage for the regions around D5S2050, D10S591 and D13S217, but not around D5S1981 or D14S288. Three-point analysis between D5S2050 and D5S400 using the CMT model produced conventional lod scores of 2.9 ($P = 0.0001$) 10 cM proximal to D5S2050 and 2.0 ($P = 0.001$) between these markers, and also an MLOD between the markers of 2.9 ($P = 0.0003$) using a dominant model with penetrance of 0.6 and phenocopy probability of 0.02. The GTS model produced a lod of 1.9 ($P = 0.002$) proximal to D5S2040 and an MLOD of 1.8 ($P = 0.004$). Three-point analysis of D10S591 and D10S189 produced lods of 1.9 ($P = 0.002$) 10–20 cM proximal to the markers with both models and an MLOD between the markers of 1.8 ($P = 0.004$) with GTS and 1.6 ($P = 0.007$) with CMT. Three-point analysis using the GTS model with D13S217 and D13S171 produced a lod of 2.7 ($P = 0.0002$) proximal to the markers and of 2.0 ($P = 0.001$) between the markers, while the MLOD midway between D13S171 and D13S218 was 2.5 ($P = 0.0007$). The CMT model produced lods of 1.1 ($P = 0.01$) and 1.7 ($P = 0.003$) with these pairs of markers.

When the pedigree was inspected visually it did seem at least possible that affection with GTS and CMT might be related to the combined effects of susceptibility loci present in these three separate regions. Some genotypes were missing, especially in all the early generations and patterns of inheritance were often not clear. However, it did appear that many affected subjects in the major part of the pedigree shared a haplotype consisting of allele 1 at D10S591 and allele 2 at D10S189, while in the remaining small part of the pedigree affected subjects often received the 1–5 haplotype. In this same small part affected subjects often shared the 2–5 haplotype of D5S2050 and D5S400, but linkage to these markers was not apparent in most of the pedigree. Finally, the 3–4 haplotype of markers D13S171 and D13S218 was present in many affected subjects scattered throughout the whole pedigree. At least one subject affected with GTS did not have any of these four haplotypes, although did have risk alleles from two of them, and could only be consistent with linkage if recombination had occurred. We regard such observations as quite subjective and not really adding to the evidence for linkage obtained from the formal statistical methods. Nevertheless, it did seem clear that there was not one single haplotype that could definitely be said to account for all or most of the cases of disease in the pedigree, and it seems very possible that more than one susceptibility allele is present, perhaps at more than one locus.

Discussion

The genome scan of this pedigree highlights three regions, on chromosomes 5, 10 and 13. The weight of evidence is about equal for each of these regions, and constitutes fairly strong support for linkage while failing to convincingly implicate any region conclusively. None of the regions overlaps with any demonstrating linkage to Tourette syndrome in previous studies, although the chromosome 5 region may coincide with that which appeared linked to the trait of hoarding in samples of sib pairs affected with Tourette syndrome (Zhang *et al.*, 2002). This finding may well simply be a coincidence.

Large pedigrees offer substantially greater power to detect linkage than samples of smaller families and sib-pairs unless the susceptibility allele is very common and especially if locus heterogeneity is present. Tourette syndrome is fairly rare and there is good evidence for a major gene effect, while the failure to produce replicated evidence for linkage to date makes it probable that more than one locus is involved. These considerations imply that large pedigrees should be useful in detecting linkage. However, as the present case illustrates, large pedigrees can bring problems of their own. Multipoint and two-locus analysis become problematic and patterns of inheritance are ambiguous in the early, untyped generations. This is especially so when the pedigree is not only large, but also complex, as with this one that contains two in-breeding loops. Perhaps more seriously, the larger the pedigree is the greater is the chance that it will contain two or more susceptibility alleles segregating independently, possibly at different loci. The probability of independent sources of disease occurring in the same pedigree is increased when, as is the case for Tourette syndrome, assortative mating is likely to take place (Kurlan *et al.*, 1994; Hasstedt *et al.*, 1995). If alleles from different loci segregate within the same pedigree then simple methods of linkage analysis that assume a major gene effect is due to the actions of a single locus will have substantially reduced power to detect linkage.

The results we have obtained seem to illustrate these problems. We do not find the kind of strongly convincing lod scores that we would expect were only a single locus to be influencing susceptibility, and we are severely limited in terms of the complexity of the analyses we can carry out. Nevertheless, we do find some evidence for linkage in each of the three regions highlighted and it does seem likely that at least one of them contains a susceptibility locus.

Another disadvantage of single large pedigrees is that although they can provide strong evidence for linkage to a

region they are not helpful for fine-mapping, and indeed once one identifies a chromosomal segment that co-segregates with disease it becomes impossible to demonstrate genetically which of many polymorphisms within that segment may be relevant. In order to fine-map susceptibility loci and eventually to identify pathogenic allelic variants association-based methods must be used. It is possible that mutations present in the pedigree we have studied are rare, or even unique, and that they may not be found in many other cases of Tourette syndrome. Nevertheless, given the weight of evidence we have obtained for the three highlighted regions, we would recommend carrying out association studies of these regions using finely spaced (0.5 cM) markers. Such studies may provide more convincing evidence for a localization and can then be followed up by more intensive studies aimed at determining polymorphisms that contribute to the genetic susceptibility to Tourette syndrome.

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Gilles de la Tourette Syndrome and Attention Deficit Hyperactivity Disorder: No Evidence for a Genetic Relationship

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Summary: Previous studies have suggested a relation between Gilles de la Tourette syndrome (GTS) and attention deficit hyperactivity Disorder (ADHD). However, the findings are inconsistent as to the exact nature of the relation. Whether the disparate findings are due to ascertainment and referral bias, the use of high-density multiply affected kindreds, and family history data is a matter of debate. We studied 40 consecutive GTS probands and their 168 first-degree relatives (FDRs) to test whether the two disorders share a common genetic mechanism. All subjects included in the study were directly interviewed. Although the rate of ADHD in the GTS probands was 40%, the rate of ADHD in the FDRs was 6%. Furthermore, the data were analyzed in two groups: relatives of probands with both GTS and ADHD and relatives of probands with GTS only. The rate of ADHD among relatives of GTS+ADHD probands was 10.3%, and the rate in the GTS−ADHD group was 4.5%. In these families of GTS+ADHD probands, no evidence suggested cosegregation of the two disorders. Our data do not support the view that ADHD may be an alternative expression of the putative GTS gene. **Key Words:** Gilles de la Tourette syndrome—Attention deficit hyperactivity disorder—Genetic mechanism—Cosegregation. **NNBN 9:192–196, 1996**

Gilles de la Tourette syndrome (GTS) is a neuropsychiatric condition characterized by multiple motor and one or more vocal tics of > 1-year duration and age of onset before age 21 years (1). The most widely used current definition of attention deficit disorder is that of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM 111R) (1), which has two diagnostic categories: attention deficit hyperactivity disorder (ADHD) and undifferentiated attention deficit disorder (UADD).

ADHD has been reported to occur in a substantial proportion of GTS patients, with rates ranging from 21 to 90% (2). However, the exact relation between the two disorders remains unclear and has been the subject of much controversy. Researchers have suggested that male GTS patients are more likely to have ADHD, that these symptoms often precede the development of tics, and that they are more pronounced in those with a severe form of GTS (3–6). Sverd and colleagues (7) suggested that increased severity of GTS symptoms is associated with an earlier age of onset of behavioral problems such as ADHD. Furthermore, in clinic samples, there may be an overrepresentation of associated problems, in particular ADHD, because of referral and ascertainment bias (8). However, the developmental course of the two disorders differs slightly. In ADHD, by definition, age of onset is <7 years. Although the subjects continue to have some attentional impairment into adult life, the symptoms mostly disappear after the early childhood years. In GTS, on the other hand, age at onset is ~7 years and the symptoms persist into adult life.

The observation that stimulants used in the treat-

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ment of ADHD cause, provoke, or exacerbate tics/GTS has led to several possible explanations regarding the biochemical relation between the two disorders. Stimulants release dopamine (DA) and norepinephrine (NE) from the presynaptic nerve terminals (9). If indeed the hypothesis of increased dopaminergic activity in GTS is correct, it is not surprising that the stimulant-induced increase in dopaminergic activity can precipitate the onset or cause exacerbation of GTS symptoms. Other evidence for such a relation derives from amphetamine-induced stereotypic behaviors in animals as well as from the occurrence of tremulousness and tics in patients taking toxic doses (10).

At a neuroanatomic level, thalamocortical pathways and frontal lobe caudate nucleus pathways have been suggested to be related to hyperkinetic symptomatology. Furthermore, a dysfunction in the dopaminergic pathways in the frontal lobe has been postulated in hyperkinesis (11). Comings and Comings (12) postulated an imbalance of mesencephalic-mesolimbic DA pathways for the pathogenesis of GTS.

Increasing evidence therefore suggests a considerable symptomatic, neuroanatomic, and biochemical overlap between GTS and ADHD. However, whether the association between the two disorders is due to such overlaps in neuroanatomically or biochemically mediated intermediate or final pathways in the genesis of clinical symptoms, or indeed is the result of a shared primary etiology, is not clear. If a genetic relation does exist between GTS and ADHD and ADHD represents an alternative phenotypic expression of the same gene, it would be that a significantly greater number of relatives of GTS patients would be expected to have ADHD. In addition, the morbid risk of ADHD should be the same in relatives of GTS+-ADHD probands, as compared with that in relatives of GTS−ADHD probands. However, if those with GTS and ADHD represent a distinct genetic subentity of the syndrome, the two conditions would cosegregate within families and both GTS and ADHD would coexist in a given individual much more often than is expected by chance alone (8).

It has also been suggested that the two disorders may share the same underlying genetic mechanism and that ADHD may represent a different manifestation of the GTS diathesis (13,14). Other investigators have refuted such an association (6,8,15). There may be several explanations for this disparity in findings. Issues relating to ascertainment and referral bias have already been discussed. In addition, studies using family history data have an inherent problem of misdiagnosis and underdiagnosis. Therefore, the true rates of illnesses among relatives may be different; consequently, the pattern of illnesses within families can be affected by such reporting bias (16,17). This is particularly relevant in the case of ADHD with an early age of onset, and most subjects reporting about their relatives may be relying on their memories or other people's accounts of their behavior during childhood. The use of multiply affected families in some studies and the fact that most probands had a severe form of the disorder are other important factors that may have influenced the findings of these studies. A much better test of genetic relation between ADHD and GTS can be accomplished with a large sample of small families that have been ascertained without regard to familial loading.

SUBJECTS AND METHODS

This study was performed with data obtained from direct clinical examination of families ascertained through 40 consecutive new cases of GTS (DSM III R), registered at the National Hospital for Neurology and Neurosurgery (NHNN), London, England, in an 8-month period. Two other patients were examined during this time, but their families were not included in this study because information was not available about the biological relatives. Of the 40 probands, 21 were aged <16 years.

All subjects underwent direct clinical examination by the National Hospital Interview Shedule (NHIS) for the assessment of GTS and related behaviors, which has been used in previous phenomenological and genetic studies (18,19). The NHIS is a semistructured interview schedule designed for use by specially trained clinicians. Subjects were interviewed in the company of other informants (typically parents or spouses). For subjects aged <16 years, a parent interview was always obtained and was combined with a clinical observation at the time of the interview. All 168 FDRs included in the study were personally interviewed by the same investigator (V.E.); 132 FDRs were interviewed at the NHNN clinic and 36 were interviewed at home. For those interviewed at the clinic, diagnosis was assigned by an independent clinician (M.M.R.) and consensus was obtained. After completion of the interview, family history data were collected in a semistructured interview from each informant about all his or her FDRs.

For a diagnosis of ADHD, information was gathered from the parent on problems relating to inattention, hyperactivity, and impulsivity according to the DSM III R criteria. The criteria items as given in the NHIS were used as a guide; in addition, the data from Family Psychiatric History (questions adapted from the Schedule for Affective Disorders and Schizophre-

nia for school age children) were used to make a diagnosis of ADHD. Thus, questions regarding inattention included information about whether the child had difficulty finishing chores, listening, and paying attention and whether the child was distractable in several different settings. For impulsivity, information was collected about the ability of the child to complete tasks, to wait his or her turn in group activities, or to think about the consequences of something before acting on it. For hyperactivity, questions were asked about whether the child "often" had trouble sitting still, and whether the child could be considered "always on the go." The term often was interpreted to the parent as behaviors occurring in >50% of the situations, and this was taken as an indication of the "pervasive" nature of the problem. The age of onset was specified as before age 7 years and duration as >6 months. Whenever possible, while interviewing adult subjects whose parents were not part of the study population, we obtained information by a telephone interview with the parent, using the Family Psychiatric History. School and teacher reports were not obtained.

These family history data were included in the final diagnostic estimates of the relatives. After interviews in a given family were completed, all available information (personal interview and family history descriptions) for each person were collated and diagnostic ratings were completed using DSM 111R criteria. Independent diagnostic estimates were performed by the two clinicians (V.E. and M.M.R.) based on all the collated information. Disagreements were resolved by a joint interview, and consensus using a "best estimate" method of diagnosis was adopted (20). If symptoms were present both on history and examination, a "definite" diagnosis was assigned. If symptoms were present on examination but supporting information from personal history and family reports was lacking, a "probable" diagnosis was given. Finally, if symptoms were present on history but were not sufficient to satisfy either a definite or a probable diagnosis, a "possible" diagnosis was given. Only definite and probable diagnoses were used in the analysis reported.

Goodness of Fit Test

FDRs were grouped according to sex of the proband, sex of the relative, and the relation to the proband (e.g., fathers of male probands, brothers of female probands). Using the goodness of fit test as incorporated in the program POINTER (21), one can calculate the expected risk of occurrence of individual and combined diagnosis of GTS and ADHD based on the best fitting genetic model [for this family data set, segregation analysis had shown that an autosomal dominant model gave the best fit (19)] and compare this with the observed rates of illnesses in the families. A statistically significant difference evident between the predicted and observed rates could be regarded as an indication to suggest poor fit for the data. If, on the other hand, for any particular clinical behavior in question, the expected and observed rates were not statistically different, this would indicate an integral part of the expression of the syndrome and hence suggesting a clinical phenotype.

To examine whether GTS and ADHD share a common genetic mechanism and whether indeed ADHD is a clinical phenotype of GTS, we analyzed the data from probands and FDRs who fulfilled the diagnostic criteria for ADHD according to DSM 111R criteria. The data were analyzed in two groups: the relatives of probands with both GTS and ADHD and the relatives of probands with GTS only. Goodness of fit test was performed with expected and observed rates as already detailed.

TABLE 1. *Rates of tics and ADHD among relatives of GTS probands*

Group	Tics (%)	ADHD (%)
Relatives of GTS+ADHD probands (n = 58)	19 (32.8)	6 (10.3)
Relatives of GTS−ADHD probands (n = 110)	32 (29.1)	5 (4.5)
Total (n = 168)	51 (30.6)	11 (6.5)

GTS, Gilles de la Tourette syndrome; ADHD, attention deficit hyperactivity syndrome.

RESULTS

Of the 40 GTS probands, 16 subjects (40%) fulfilled the diagnostic criteria for ADHD. The M/F ratio of the probands was 3:1. In all, 168 relatives (M 90, F 78) were studied: 30 (17.9%) had GTS, 21 (12.5%) had chronic multiple tics (CMT), and 11 (6.5%) had ADHD. When we divided the data into two groups (relatives of GTS+ADHD probands and relatives of GTS−ADHD probands) we noted that the occurrence of ADHD was more than double in the former group as compared with the latter (10.3 vs. 4.5%), although this did not reach statistical significance, whereas the occurrence of tics did not differ (32.8 vs. 29.1%) greatly between the two groups (Table 1). Males comprised 81% of the GTS+ADHD probands and 70% of the GTS−ADHD probands.

TABLE 2. *Frequency of GTS, CMT, and ADHD among relatives of GTS probands*

Diagnosis	n	Frequency
GTS only	23	0.137 ± 0.034
CMT only	20	0.119 ± 0.031
ADHD only	3	0.012 ± 0.014
GTS+ADHD	7	0.041 ± 0.023
CMT−ADHD	1	0.006 ± 0.003

CMT, chronic multiple tics; other abbreviations as in Table 1.

Table 2 shows the frequency of GTS, CMT, and ADHD among all 168 FDRs; Table 3 shows the frequency separately for GTS+ADHD probands and GTS−ADHD probands. The frequencies were calculated with the computer programme POINTER (21). Only three individuals had ADHD without some tic disorder. Furthermore, although most probands diagnosed as having GTS+ADHD were rated as moderate to severe (mild = 12.5%, moderate = 56%, severe = 31.5%) on the clinician's rating (based on the impairment of functioning and need for medication) and the Yale Global Tic Severity Scale (22), those with GTS−ADHD more often received a mild to moderate rating (mild = 40%, moderate = 31%, severe = 29%).

To test whether GTS and ADHD were cosegregating within families, we studied the association of GTS and ADHD in the affected relatives of GTS+ADHD probands. If cosegregation exists, there should be a nonrandom association different from that predicted. The observed and expected risks of being affected with individual (GTS, CMT, ADHD) and combined diagnosis (GTS+ADHD and CMT+ADHD) are shown in Table 4. Goodness of fit chi-square test showed that the expected and observed rates were not significantly different, suggesting that the association is in no way different from that expected by chance alone.

DISCUSSION

Our results support the fact that the rate of occurrence of ADHD is increased in GTS probands (40%), which is in keeping with results of earlier studies. Although the M/F ratio of the total sample of probands was 3:1, there were proportionately more males in the GTS+ADHD group, as expected. However, only 6.5% of the FDRs qualified for a diagnosis of ADHD. Because there were no control subjects in the study, it is difficult to compare this percentage with the available general population prevalence, estimated to be 3–10% (23).

TABLE 3. *Frequency of GTS, CMT, and ADHD among relatives of GTS+ADHD probands as compared with GTS−ADHD probands*

Diagnosis	Relatives of GTS+ADHD probands	Relatives of GTS−ADHD probands
GTS	13/58 (0.224)	17/110 (0.154)
CMT	6/58 (0.103)	15/110 (0.136)
ADHD	6/58 (0.103)	5/110 (0.045)

Abbreviations as in Tables 1 and 2.

TABLE 4. *Expected and observed number of relatives in families of probands with GTS+ADHD*

Diagnosis	Expected	Observed
GTS	6.02	9
CMT	4.20	6
ADHD	2.18	2
GTS+ADHD	2.38	4
CMT+ADHD	0.32	0
None	42.89	37

Abbreviations as in Tables 1 and 2.
Chi-square = 4.502.

Between-group comparisons showed a much higher occurrence of ADHD among relatives of GTS+ADHD probands. There are different ways of explaining this finding. First, the two disorders are not genetically related. Second, GTS+ADHD represent a distinct genetic subtype, and they cosegregate within families. However, our findings from the goodness of fit test suggest that the two disorders appear to segregate independently. Third, GTS+ADHD may be a clinically separate group representing a more severe form of the disorder with more extensive involvement of neurochemicals and neuroanatomic structures and thus possibly causes greater risk of exhibiting ADHD symptoms. Our finding that the GTS+ADHD subjects had a more severe form of the disorder tends to support this view. Age and sex may be other modifying factors. Very few of our probands in the GTS+ADHD group were females, thus precluding any meaningful comparisons between groups based on the sex of the proband. If indeed an association exists between age of onset, sex, and severity, the referral bias in clinic samples may be further compounded.

The finding that only three relatives had ADHD in the absence of tics is of interest. If ADHD were an alternative expression of the same gene, one would expect more relatives to have ADHD, in the absence of any tics. However, ADHD may well be one aspect of the GTS phenotype, mediated by an overlap in the intermediate or final pathways of expression, rather than an alternative expression. The overlap could exist at a biochemical (common neurotransmitter involvement such as DA and NE), neuroanatomic (both basal ganglia and frontal lobe have been implicated in both the disorders), or symptomatic level. For exam-

ple, children with GTS, because of their involuntary movements, may appear fidgety and overactive. In addition, if they try to suppress their tics, they may experience mounting inner tension, which in turn may affect their ability to attend to and concentrate in tasks. Therefore ADHD occurring in the context of GTS may be the result of different mechanisms, varying from being a clinical aspect of GTS itself, being secondary to the GTS symptoms; being a behavior that forms a final common pathway for a variety of conditions in which frontal lobe and basal ganglia are involved, of which GTS is one; or being a comorbid condition exaggerated by referral and ascertainment bias. This may also be the result of Berkson effect (24), by which, for statistical reasons separate from referral bias, the comorbidity rate in clinical samples will always be greater than that in the general population whenever only a small proportion of the conditions constituting the comorbidity pattern are referred to the clinics. Other explanations include shared and overlapping risk factors and diagnostic considerations.

Our failure to detect cosegregation will have to be interpreted cautiously because of the small sample size. However, because our findings are not significant, the power of the analysis is estimated to be 0.78 ($df = 5$; $\alpha = .33$; $\beta = .22$). Other limitations of our study include lack of a control group to allow calculation of the base rates of ADHD. Furthermore, there was no ADHD proband group with which to compare the rate of GTS in the FDRs. In addition, the interviewer was not blind to the GTS diagnosis of the proband. However, we do believe this has not biased our findings because the main focus was the difference in rates between relatives of GTS+ADHD and GTS−ADHD probands, and although not blind as to the GTS status of the proband, the investigator was not aware at the time of interviewing the family members of whether the proband also had a diagnosis of ADHD.

Further studies are indicated with a larger sample size, using normal subjects and ADHD probands as controls, and taking into account some of the relevant patient characteristics such as the age of onset of tics and ADHD, sex of the proband, and severity of the disorder.

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ed may represent one critical period when ge- information is "tagged" or marked, and methyl- may be the molecular mechanism involved in orarily changing the genetic information to per- differential expression.[2] This is contrary to the lelian tenet that the parental source of genetic mation does not influence gene expression. mic imprinting, however, appears to be a form gulation allowing another level of flexibility in control of expression of the human genome.

enomic imprinting is found in several genetic ders, including Prader-Willi and Angelman syn- es,[3] bilateral retinoblastoma,[4] myotonic dystro- [5] and Huntington's disease.[6] Other examples re the sex of the transmitting parent is shown to ence the severity or nature of the clinical ex- sion include neurofibromatosis types 1 and 2,[7,8] bellar ataxia,[9] seizures,[10] spinocerebellar atax- and Fragile X syndrome.[12] Furthermore, if there two loci for a disorder, there may be two different s of imprinting, as might be found with tuberous rosis and adult polycystic kidney.[2] However, for y genetic disorders, there are no data on the erential expression of specific genes when inher- from the father versus the mother. The role of der in parental origin is particularly relevant in case of Tourette's syndrome (TS), because there known sex effects observed in this disorder; for mple, females more often have an obsessive com- sive expression of the disorder and there are more cted males than females.[13,14]

thods. The families for this study were identified ugh the TS clinic at the National Hospital for Neurol- and Neurosurgery, Queen Square, London, U.K. In ition to the 168 First Degree Relatives (FDRs) ascer- ed through 40 consecutive TS probands for whom seg- ation analysis had indicated an autosomal dominant smission,[15] the study also included 229 family mem- s from 17 multigenerational TS pedigrees (where there e more than five affected family members) chosen for purpose of linkage analyses.[16] Direct clinical examina- was carried out in all the subjects using the National spital Interview schedule for the assessment of TS and ated behaviors. This is a semi-structured interview edule that has been used in previous genetic studies[14-16] uses the best estimate diagnosis method.[17] The reli- lity and validity of this instrument are established.[18] Subjects were interviewed in the company of other in- mants, typically parents or spouses. For those subjects der age 16, a parent interview was always obtained, and s was combined with the clinical observation at the time the interview. For those subjects above age 16 whose rents were not part of the study population, information out childhood was obtained by a telephone interview. mily history data were collected from each informant out all FDRs, and were included in the final diagnostic imates of each of the relatives. Thus, following the com- tion of all interviews within a given family, all available formation (personal interview and family history de- iptions) for each individual was collated and diagnostic tings were completed using DSM IIIR criteria.[19] When

Table Rate of occurrence of TS, CMT and OCB in offspring of transmitting males and females

	TS N (%)	CMT N (%)	OCB N (%)
Paternal transmission	50/77 (50%)	70/77 (90.9%)	26/77 (33.6%)
Maternal transmission	60/80 (75%)	77/80 (96.2%)	29/80 (36.2%)
p with 1 df	0.168	0.172	0.744

TS = Tourette's syndrome; CMT = chronic motor tics; OCB = obsessive compulsive behavior; df = degree of freedom.

symptoms were present both on history and on examination, a "definite" diagnosis was assigned. If symptoms were present on examination but there was lack of supporting information from personal history and family reports, a "probable" diagnosis was given. Finally, if some symptoms were present on history but not enough to satisfy either a probable or definite diagnosis, a "possible" diagnosis was given. Only definite and probable diagnoses were used in the analyses reported here.

Age at onset was defined in terms of the onset of first symptom (motor tic, vocal tic, or obsessive compulsive behavior) and where possible, this was corroborated by the parent or relatives. The phenotypic definitions used in the analyses were TS, chronic motor tic, and obsessive compulsive behavior (based on the consensus from available literature). The age at onset, age at diagnosis, and phenotypic expressions in the offspring of affected males were compared with that of the offspring of affected females. Data were verified after entry into a database and t test and chi-square analyses were done using SPSS/PC (SPSS Inc., Chicago, IL).[20]

Results. Of the total 437 subjects, 73 cases (16.7%) demonstrated evidence of maternal transmission and 61 cases (13.9%) demonstrated paternal transmission. The offspring showing evidence of maternal transmission showed earlier age at onset (mean = 7.04; SD = 3.05) when compared with offspring showing evidence of paternal transmission (mean = 8.50; SD = 3.79), which was statistically significant ($t = -2.43$; $df = 132$; two-tailed $p < 0.017$). We performed the Mann-Whitney U test on the age at interview to check whether this finding was due to a bias through sampling younger or older people in either of the two groups. The age distribution was not significantly different (two-tailed $p = 0.3379$) between the two groups. Chi-square analyses (chi-square for heterogeneity) of the different phenotypic definitions and sex of the transmitting parent failed to provide evidence of significant group differences (table). In addition, there were no significant differences between the two groups regarding the age at diagnosis.

Discussion. If the age at onset of symptoms in TS is determined by an age-dependent methylation of the putative TS gene or some other chronogenetic mechanism,[21] then the effect must stem from early embryogenesis. In adult polycystic kidney disease[22] some linkage data suggest that there are two or more linkage groups, and that there is an earlier age at onset of the disease with maternal transmission

for one group, and with paternal transmission for the other. If a similar mechanism is in operation in TS, perhaps there is more than one locus involved in causing distinct subtypes. This could be considered as one of the reasons for the failure so far to establish linkage in TS. Another plausible explanation of the finding may be that intrauterine environmental influences may act to produce the early onset of TS symptoms in those individuals carrying the putative gene. Such a mechanism is shown to be in operation in myotonic dystrophy.[23] Possible candidates for such an influence in TS include perinatal events, exposure to chronic intermittent psychosocial stress, exposure to thermal stress, exposure to androgenic steroids, and exposure to cocaine or other stimulants.[24]

In this study, there were no between-group differences with regard to age at diagnosis or phenotypic expressions. The latter finding is in keeping with the study by Furtado and Suchowersky,[25] who failed to find any significant differences in the age at onset or the frequency of occurrence of various TS-related symptoms between maternally and paternally transmitted cases. However, that study was limited because of the small sample size, and the use of a retrospective medical chart review method; we suspect that the information in the medical charts would be particularly unreliable for the age at onset. In our study, we believe that a more accurate estimate of the age at onset and diagnostic status of the relatives has been achieved by using the direct interview method coupled with the family history data. The variable expressivity of TS could camouflage mild cases if they are not clinically examined. Therefore, we included in the analysis only those subjects who were available for examination. To overcome any bias due to a child's knowledge of minor symptoms in a mother compared to a father, we used multiple informants, and the information given by all other family members about a given subject was used in arriving at the best estimated diagnosis. Although the clinical rater was not blind to the family history, thus adding an element of bias in the ratings, this is unlikely to influence the information about the age at onset, which is the main positive finding in this study. However, the sex of the affected parent could be a confounding factor in the reporting of age at onset. For example, a mother who is affected and hence familiar with TS symptoms may identify early manifestations of the condition in her offspring, compared with an affected father, who may not notice manifestations in offspring as early.

Lichter et al.,[26] using family history methodology, found that maternal transmission was associated with greater motor tic complexity and more frequent non-interfering rituals, while paternal transmission was associated with increased vocal tic frequency and more prominent attention-deficit hyperactivity disorder (ADHD) behaviors. In our study, we did not use ADHD as a possible phenotypic expression, given the controversy surrounding this issue.[27-31]

The pedigree of a disease gene, and its mutation that is imprintable, can appear to show autosomal dominant, autosomal recessive, or multifactorial inheritance, depending on which part of the family is being observed.[32] Thus, the findings from this study indicate that there is a need to re-examine family data separately for maternally versus paternally transmitted cases.

If this finding can be replicated consistently, the earlier age at onset found in maternally transmitted cases offers a new dimension for further investigations, as it suggests a new level of control in the nature and expression of the putative TS gene. At present, however, only a small percentage of families seems to manifest this differential effect, suggesting that if these are imprinting effects, they do not occur in all families or between all chromosome parts. Nevertheless, the trend towards differential expression when inherited primarily from the male or female parent poses a new challenge and raises the possibility of imprinting as one of the modifying factors in the genesis and expression of TS.

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Bilineal transmission in Tourette's syndrome families

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Article abstract—We assessed the frequency of bilineal (from maternal *and* paternal sides) transmission of Tourette's syndrome (TS) in two groups of pedigrees: (1) 39 high-density families in which five or more relatives were reported to have TS, and (2) the families of 39 consecutively ascertained probands referred for evaluation of TS. We used two designations for the TS phenotype (tics, tics or obsessive-compulsive behavior [OCB]), and we attempted to verify bilineal transmission with direct examinations. For the high-density pedigrees, bilineal transmission was evident in 33% (considering tics) and 41% (considering tics or OCB) of families, which was confirmed by examination in 77% of the kindreds. For the consecutive pedigrees, bilineal transmission was seen in 15% (tics) and 26% (tics or OCB) of families, which was verified by examination in 66% of the kindreds. Both parents of the proband were affected (tics or OCB) in 38% of the high-density pedigrees and 10% of the consecutive pedigrees. For the high-density families only, the frequency of bilineal transmission appeared to be related to the proband's severity of TS, and for both pedigree groups, the frequency of both parents being affected was higher in families in which the proband's symptoms were most severe. Our findings support the contention that bilineal transmission and homozygosity are common in TS. These genetic phenomena might play a role in determining severity of illness and may explain current difficulties in localizing the gene defect by linkage analysis.

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Although Gilles de la Tourette described the familial nature of the condition he reported in 1885, it was not until the late 1970s that studies demonstrated an increased frequency of tics in the families of Tourette's syndrome (TS) patients.[1-3] Analysis of family history data by Pauls et al[4] supported vertical transmission of tics, thereby suggesting a hereditary etiology for the disorder. Five separate studies used family history information to test specific genetic hypotheses regarding the hereditary transmission pattern of TS.[5-9] All studies reported that the pattern of inheritance was consistent with

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a single gene of major effect; three of the studies supported an autosomal dominant model,[5,7,9] and two could not reject a hypothesis of multifactorial-polygenic transmission.[6,7] Data obtained from direct examinations of the first-degree relatives of TS probands were subsequently used for segregation analyses, which supported autosomal dominant inheritance and indicated that chronic tic disorder and obsessive-compulsive disorder are variant expressions of the syndrome.[10] The most recently performed segregation analysis studies continue to support an autosomal dominant transmission pattern in studied families.[11,12]

In 1989, Comings et al[13] reviewed 170 TS pedigrees and found that in 8.2% of the families there was a history of tics on both the maternal and paternal sides. In addition, behaviors potentially associated with TS, including obsessive-compulsive behavior (OCB), panic attacks, attention deficit hyperactivity disorder, and severe alcohol or drug abuse, were present on both sides for 34.7% of the families. Based on these observations, the authors suggested that many individuals with TS may be homozygous for the disease gene defect. Furthermore, they proposed that the hereditary transmission pattern for TS can be best described as "semi-dominant, semi-recessive," whereby clinical expression is determined by whether an individual inherits one or two doses of the TS gene (ie, TS is a dominant trait with a dosage effect). This work has not received wide acceptance since the data was based on patient self-report questionnaires rather than direct clinical interviews, and since the authors included wide-ranging psychopathology as part of the TS clinical spectrum, for which there is currently insufficient support.[14]

In the course of evaluating and caring for several hundred patients with TS, it has been our clinical impression that the symptoms of TS are indeed commonly reported on both the maternal and paternal sides in affected families. In order to investigate the frequency of such bilineal transmission, we studied two groups of TS pedigrees collected at the National Hospital for Neurology and Neurosurgery (NHNN), Queen Square, London. The first group included a series of high-density pedigrees in which five or more family members were reported to be affected. The second group consisted of the families of probands consecutively evaluated at NHNN in 1991. The nuclear families of these probands had been previously examined as part of a segregation analysis study.[11] For both family groups, we employed a definition of disease phenotype that included as affected only individuals with tics or OCB. In addition, we attempted to verify the presence of symptoms on both parental sides through direct clinical examination of family members.

Methods. We included 39 high-density pedigrees in which five or more relatives within three generations of the proband were reported to have tics or OCB (lifetime history). The probands of these families were evaluated at NHNN between 1989 and 1993. We also studied 39 pedigrees of probands consecutively evaluated at the NHNN during an 8-month period in 1991. For all families, historic information was obtained from the proband and relatives (parents of children, spouses of adults) who were present at the time of the clinical interview. Data were collected in a standardized format using the National Hospital Tourette Questionnaire. This instrument includes specific questions regarding the presence of tics and OCB (as well as other symptoms associated with TS) in family members. In each family, we attempted to document the presence of tics by direct clinical examination for at least one member of the maternal side and one member of the paternal side. If subjects reported to have tics were unavailable, we then carried out direct clinical interviews for the presence of OCB using the Leyton Obsessional Inventory[15] in relatives reported to have these symptoms. In some families, such targeted relatives were unavailable or unwilling to participate for such direct assessments. For each of the consecutive pedigrees, the proband and available siblings and parents had previously been assessed with direct clinical evaluations as part of a segregation analysis study. In these families, we carried out additional clinical interviews for pertinent family members in order to verify bilineal transmission. For both pedigree groups, our definition of bilineal transmission was restricted to include relatives within three generations of the proband. No consanguinity was evident in any of the families. For each family, we rated the severity of TS in the proband as mild (not interfering with daily functioning, not requiring medications), moderate (some functional impairment, requiring medications), or severe (substantial functional impairment, requiring medications).

A two-sided Fisher's exact test, modified using the mid-p value,[16] was used to compare the high-density and consecutive families regarding the proportion of families having bilineal transmission. The same test was used to compare the high-density and consecutive families regarding the proportion of probands having both parents affected. These analyses were performed using two different definitions of phenotype: tics alone, and either tics or OCB.

The Cochran-Armitage test[17] was used to examine the hypothesis that the proportion of families having bilineal transmission (tics or OCB) increases with the severity of the proband. This was done separately for the high-density and the consecutive families. The same test was used to examine the hypothesis that the proportion of probands having both parents affected (tics or OCB) increases with the severity of the proband. The exact permutation distribution of the test statistic was used for computing p values.

All tests were performed using the StatXact statistical software package.[18]

Results. *High-density pedigrees.* The clinical features of the 39 high-density pedigrees are summarized in table 1. For this group, 44 of the siblings or children of the probands were reported to be affected by tics or OCB. Since these individuals could not be assigned to either maternal or paternal sides, they were excluded from the analysis of bilineal transmission. On the probands' maternal side, 51 relatives were reported to have tics, 33 OCB, and 18 both tics and OCB. For these subjects, 15

Table 1. Clinical features of 39 high-density pedigrees

No.	Severity of proband	Unilineal or bilineal (maternal/paternal)	Maternal			Paternal		
			Tics	OCB	Both	Tics	OCB	Both
1	Severe	B* (B/B)	2/0	1/0	1/1	1/0	1/0	1/1
2	Moderate	U	0	0	0	3/0	0	2/1
3	Mild	B (T/B)	4/1	0	0	1/0	1/0	3/1
4	Severe	B (T/B)†	0	0	0	7/4	0	1/1
5	Mild	B* (B/T)	1/0	1/1	0	1/1	0	0
6	Severe	B* (B/B)	3/3	4/4	0	3/3	2/2	0
7	Moderate	B (B/B)	0	0	1/1	1/0	0	1/0
8	Moderate	B (B/B)	1/0	1/0	0	0	0	1/0
9	Severe	B* (B/B)	1/0	2/0	1/1	1/1	1/0	0
10	Severe	B (O/B)	0	3/0	0	0	6/0	1/0
11	Moderate	U	6/6	3/3	0	0	0	0
12	Mild	U	0	0	0	2/0	1/1	2/1
13	Mild	U	1/0	1/0	2/1	0	0	0
14	Severe	B* (B/B)	4/1	1/0	0	0	0	1/1
15	Severe	B* (B/T)	0	0	1/1	2/1	0	0
16	Mild	U	1/0	0	1/0	0	0	0
17	Mild	U	3/0	0	1/0	0	0	0
18	Mild	U	4/0	0	1/0	0	0	0
19	Mild	U	2/0	0	0	0	0	0
20	Mild	U	3/0	0	1/0	0	0	0
21	Mild	U	0	2/0	1/1	0	0	0
22	Moderate	B* (B/B)	1/0	3/0	1/1	1/0	2/0	1/1
23	Severe	U	0	0	0	3/1	0	1/0
24	Moderate	U	0	0	0	1/0	0	0
25	Mild	U	3/1	1/0	1/1	0	0	0
26	Mild	B (O/B)	0	1/0	0	1/0	1/0	1/1
27	Moderate	U	0	0	0	1/0	1/1	2/1
28	Moderate	B* (B/B)	2/1	2/0	0	8/2	3/0	1/1
29	Mild	U	1/0	5/0	3/1	0	0	0
30	Moderate	B* (O/T)	0	0	1/1	2/1	0	0
31	Mild	U	0	2/0	1/1	0	0	0
32	Moderate	U	0	0	0	4/0	0	0
33	Severe	U	0	0	0	1/1	1/0	1/0
34	Severe	B* (T/B)	2/1	0	0	0	0	2/1
35	Severe	U	2/0	0	0	0	0	0
36	Mild	U	4/1	0	0	0	0	0
37	Mild	U	0	0	0	1/0	1/0	0
38	Moderate	U	0	0	0	1/0	2/0	0
39	Mild	U	0	0	0	1/0	1/0	1/1
Totals	Mild = 17 Moderate = 11 Severe = 11	B = 16 (41%) U = 23 (59%)	51/15 (29%)	33/8 (24%)	18/11 (61%)	47/15 (32%)	24/4 (17%)	23/12 (52%)

OCB Obsessive-compulsive behavior.
† Married-in spouse affected.

For unilineal (U) or bilineal (B) expression, an asterisk indicates that bilineality was verified by direct examination. In parentheses, we indicate the clinical phenotype (T = tics, O = OCB, B = both tics and OCB) observed on the maternal/paternal sides. For the maternal and paternal sides, the numerator indicates the number of relatives reported to be affected by tics, OCB, or both, and the denominator indicates how many of these subjects were examined.

(29%) with tics, eight (24%) with OCB, and 11 (61%) with both tics and OCB were directly examined by the investigators. On the paternal side, 47 subjects were reported to have tics, 24 OCB, and 23 both tics and OCB. For these subjects, 15 (32%) with tics, four (17%) with OCB, and 12 (52%) with both tics and OCB were examined. In total, 65 of the 196 relatives (33%) reported to have tics or OCB were examined. In these 39 high-density families, bilineal transmission was found in 13 kindreds (33%) when tics alone was considered the affected phenotype. The number with bilineal transmission increased to 16 (41%) when the phenotype was extended to include tics or OCB. The presence of tics in at least one member of the maternal and paternal sides was confirmed by direct examination in 10 (77%) of the families with reported bilineal transmission of tics. In the high-density pedigrees, both parents were affected in 12 kindreds (31%) when tics alone was considered and in 15 kindreds

Table 2. Clinical features of 39 consecutive pedigrees

No.	Severity of proband	Unilineal or bilineal (maternal/paternal)	Maternal			Paternal		
			Tics	OCB	Both	Tics	OCB	Both
1	Severe	U	0	0	0	1/1	0	0
2	Mild	B* (B/O)	1/1	1/0	0	0	2/0	0
3	Mild	B (T/B)	1/0	0	0	1/1	0	1/0
4	Mild	U	0	0	0	1/0	0	0
5	Moderate	U	2/1	0	0	0	0	0
6	Severe	B* (O/B)	0	3/1	0	1/0	1/0	1/1
7	Severe	B (T/B)	1/0	0	0	0	1/0	1/1
8	Severe	B (B/T)	1/0	1/0	0	3/1	0	0
9	Severe	U	0	0	0	3/0	1/0	2/0
10	Mild	U	1/1	0	0	0	0	0
11	Moderate	U	1/1	0	0	0	0	0
12	Moderate	U	1/1	0	0	0	0	0
13	Mild	B (B/B)	5/0	1/0	0	2/0	0	1/1
14	Severe	U	0	0	0	0	1/0	0
15	Moderate	B (B/B)	2/0	0	1/0	0	1/0	2/1
16	Severe	U	0	0	0	1/1	0	0
17	Mild	B (B/O)	0	0	1/1	0	1/0	0
18	Mild	U	0	0	0	4/0	0	1/1
19	Mild	U	0	0	0	1/1	0	0
20	Severe	U	0	0	1/1	1/0	1/1	0
21	Severe	B* (O/B)	0	1/1	0	0	0	1/1
22	Severe	N	0	0	0	0	0	0
23	Mild	B* (B/T)	2/1	0	1/0	1/1	0	0
24	Severe	U	1/0	0	0	0	0	0
25	Mild	U	0	0	0	4/1	0	0
26	Severe	U	3/2	0	0	0	0	0
27	Moderate	U	1/1	0	0	0	0	0
28	Moderate	U	2/1	1/0	0	0	0	0
29	Mild	U	3/0	1/1	0	0	0	0
30	Moderate	U	1/1	0	0	0	0	0
31	Moderate	U	0	0	0	1/0	0	0
32	Moderate	U	1/1	0	0	0	0	0
33	Severe	U	4/1	0	0	0	0	0
34	Severe	U	0	1/1	0	0	0	0
35	Moderate	U	2/1	0	0	0	0	0
36	Severe	U	2/1	0	0	0	0	0
37	Moderate	U	0	0	0	2/0	0	0
38	Severe	U	0	0	0	1/1	0	0
39	Moderate	U	1/1	0	0	0	0	0
Totals	Mild = 11 Moderate = 12 Severe = 16	B = 10 (26%) U = 28 (72%) N = 1 (2%)	39/16 (41%)	10/4 (40%)	4/2 (50%)	28/8 (29%)	9/1 (11%)	10/6 (60%)

OCB Obsessive-compulsive behavior.
N No affected relatives.

For unilineal (U) or bilineal (B) expression, an asterisk indicates that bilineality was verified by direct examination. In parentheses, we indicate the clinical phenotype (T = tics, O = OCB, B = both tics and OCB) observed on the maternal/paternal sides. For the maternal and paternal sides, the numerator indicates the number of relatives reported to be affected by tics, OCB, or both, and the denominator indicates how many of these subjects were examined.

(38%) when the TS phenotype included tics or OCB. Such mating of two affected individuals was also observed in the grandparents of probands in 11 instances. In addition, in two cases, individuals who married into the family were reported to have TS, including tics (one case) and OCB (one case).

Consecutive pedigrees. The clinical features of the 39 consecutive families are summarized in table 2. As expected, the numbers of relatives reported to be affected by TS were lower than in the high-density pedigrees. Twenty-four siblings or children of probands were reported to be affected by tics or OCB, and they were not included in our analysis of bilineal transmission. On the maternal side of the probands, 39 relatives were reported to have tics, 10 OCB, and four both tics and OCB. On the paternal side, 28 were reported to have tics, nine OCB, and 10 both tics and OCB. Of these subjects, 16 (41%) with tics, four (40%) with OCB, and two (50%) with both tics and OCB on the maternal

Table 3. Frequency of bilineal transmission and frequency of both parents being affected according to severity of proband

	High density (N = 39)		Consecutive (N = 39)	
	Bilineal transmission*	Both parents affected*	Bilineal transmission†	Both parents affected‡
Mild	3/17 (18%)	3/17 (18%)	5/11 (45%)	1/11 (9%)
Moderate	5/11 (45%)	5/11 (45%)	1/12 (8%)	0/12 (0%)
Severe	8/11 (73%)	7/11 (64%)	4/16 (25%)	3/16 (19%)

The number (percent) of families with bilineal transmission or both parents being affected (including tics or OCB) is shown for each of three levels of Tourette's syndrome severity (mild, moderate, severe) for the proband.

* $p < 0.003$ for Cochran-Armitage test for trend.
† p = not significant.
‡ $p = 0.25$.

side underwent direct clinical examinations. Eight subjects (29%) with tics, one (11%) with OCB, and six (60%) with both tics and OCB on the paternal side were examined. In total, 37 of the 100 relatives (37%) reported to have tics or OCB were examined. In the 39 consecutive pedigrees, bilineal transmission was less evident ($p < 0.07$) than in the high-density pedigrees, being observed in six kindreds (15%) when tics was considered to be the disease phenotype and in 10 kindreds (26%) when the phenotype was extended to include tics or OCB ($p < 0.16$). The presence of tics in at least one member on each side was confirmed by direct examination in four of the six families (66%) reporting bilineal expression of tics. We found that both parents were affected by tics in only one (3%) of the consecutive families and by tics or OCB in four families (10%). These rates were significantly lower than those observed in the high-density pedigrees ($p = 0.0008$ for tics, 0.007 for tics or OCB).

Severity of TS. For the high-density pedigrees, we found a trend ($p < 0.003$) indicating that the frequency of bilineal transmission is related to the severity of TS in the proband (table 3). Bilineal transmission was found in 18% of families with mild severity, 45% with moderate severity, and 73% with severe symptoms. This relationship was not evident for the consecutive pedigrees. For both groups of pedigrees, we identified a trend ($p = 0.003$ for high-density families, 0.25 for consecutive families) suggesting that the frequency of both parents being affected is also related to the severity of TS in the proband, with the higher frequency associated with more severe symptoms (table 3).

Discussion. Our initial information regarding bilineal transmission of TS was generated by the family history method, which depends on historic information provided by relatives. It has been our experience in studying large TS kindreds for linkage analysis that when family members indicate a certain relative is affected, their assessment is usually correct. In fact, family members tend to significantly underestimate the number of affected relatives when compared with diagnoses established by investigator examinations.[19] Using the family study approach, we were able to verify the presence of tics in at least one member of the maternal and paternal sides by direct examination in 77% of the high-density pedigrees and 66% of the consecutive families. Our study is limited, however, by the fact that we did not examine all relatives in the pedigrees of interest. In this case, there may have been some false-positive diagnoses based on family reports as well as false-negative diagnoses for relatives whose symptoms of TS were overlooked by the historians. Pauls et al[20] have shown that when relatives are examined directly, the recurrence risks and patterns within families can change when compared with family history data. Our study also includes other potential shortcomings. First, for some subjects, sufficient historic and examination data were not obtained in order to make assignments to specific tic disorder diagnostic categories (eg, TS, chronic multiple tic disorder). Most studies do, however, indicate that the range of primary tic disorders does fall within the TS clinical spectrum.[10,21,22] Second, for many subjects, the diagnosis of OCB was made on clinical grounds and not substantiated with standardized rating scales or formalized structured psychiatric interviews. There is currently no substantial information regarding the issue of whether the diagnosis of TS is more or less secure for subjects who have both tics and OCB versus those with one type of symptom only.[23] Third, due to ascertainment bias, our analysis may overestimate the frequency of bilineal transmission in TS families in general since our probands consisted of patients with TS whose symptoms were severe enough that medical attention was sought. Fourth, our study did not include a control group to clarify the unknown probability that any given person, regardless of their own history of tics, may have a family history of tics. Finally, we did not include any kind of age correction in our analyses; it is possible that for families with more young (child) subjects, tics or OCB might not yet have been expressed and this situation could therefore have affected our assessment of bilineal transmission. Given these limitations, our study is viewed as a pilot, preliminary investigation of bilineal transmission in TS families.

Our study does represent the first one to examine the bilineal transmission of TS using a strict definition of disease phenotype and that attempted to confirm affected status using direct clinical examinations. We found evidence of bilineal transmission in 33% of the high-density families and 15% of the consecutive pedigrees when including tics only, thus yielding figures significantly higher than the 8.2% observed by Comings et al.[13] When expanding the phenotype to include tics or OCB, we found that 41% of the high-density families and 26% of the consecutive families showed bilineal transmission. These figures, which are based on a conservative designation for the behavioral compo-

. of the TS spectrum (OCB only), compare with 34.7% rate of bilineal transmission observed by ıings et al[13] when a wider range of psy- ıathology was included. We found that both ınts were affected by tics in 31% of the high- ity families and 3% of the consecutive families by tics or OCB in 38% of the high-density pedi- s and 10% of the consecutive pedigrees. These res compare with the rates of both parents g affected by tics in 4.7% and tics or associated viors in 19.4% as reported by Comings et al.[13] , our findings support the contention of Com- et al[13] that bilineal transmission is common in amilies, even when a narrower definition of se phenotype is used, and that since both par- are often affected, many cases of TS are proba- ıomozygous for the genetic trait. However, it is ible that some affected relatives manifested ptoms on a nongenetic basis (ie, as pheno- s). Our observations indicating that the fre- cy of bilineal transmission and of both parents g affected are higher in families with the most rely affected probands also supports the hy- esis of Comings et al[13] that a genetic dosage ef- may be important in influencing the clinical ession of TS.

ur observed rates of bilineal transmission in high-density pedigrees of 33% (for tics) and (for tics or OCB) are higher than the rates for consecutive families (15% for tics, 26% for tics CB). This finding suggests that the bilineal oc- nce of TS contributes to the high density of af- d cases observed in many families. This notion pported by the large high-density TS kindreds have been employed for international efforts to ize the TS genetic defect by linkage analysis. ır own Canadian Mennonite pedigree, for ex- le, both parents of the proband have clinical nce of tics.[24] In the same pedigree, there is one documented instance of mating between two ted family members. Evidence for such mating ected individuals has been found in eight of 17 ıs in a large fundamentalist Mormon pedigree g investigated by McMahon et al.[25] Five in- es of matings of affected individuals have identified by Sandor et al (personal communi- n) in a group of TS kindreds under study in to. The selection of kindreds known to have a density of affected members, which are typi- sought for linkage analysis studies, may make re likely to encounter bilineal transmission.

e use of bilineal (intercross) families for link- nalysis is satisfactory as long as the mode of itance is clear and the disease is not charac- d by genetic heterogeneity within families.[26,27] ikelihood of genetic heterogeneity should be in genetic isolates, such as the Canadian onite kindred, and a two-locus model can be for the analysis of bilineal families.[28] Never- s, linkage studies are critically dependent on correct parameters of gene frequency, mode eritance, and penetrance. The existence of bi- lineal transmission in TS kindreds with frequent homozygosity could have a major deleterious impact on linkage analysis, particularly if genetic heterogeneity is present. Such bilineal pedigrees violate the assumptions of standard one-trait-locus models and complicate the analysis. This situation represents one possible explanation for the unsuccessful localization of the TS genetic defect to date.[29] Under these circumstances, sib-pair analysis may be a better technique for studying linkage in TS, but even the power of this approach would be reduced if, in a number of families, one of the parents was homozygous for the TS gene.[13]

If most TS patients are homozygotes and there is a single major locus, then the gene frequency can be calculated as the square root of the frequency of TS in the population.[13] Recent epidemiologic studies have suggested that the prevalence of TS is much higher than previous estimates.[30,31] High carrier frequencies raise the possibility that there may be a variety of genetic mechanisms that can produce the same phenotype. Thus, genetic heterogeneity is expected that would preclude the combining of linkage exclusion data across different families, as has been done,[32] and that would predict major difficulties for the linkage analysis approach to localizing the gene defect or defects. A very high gene (disease) frequency might explain the frequent mating of affected individuals seen in TS families, but there may be other currently unidentified reasons for this observation. The phenomenon of assortative mating (nonrandom mating in which spouses choose one another based on some characteristics associated with a particular genotype) may be at play. For example, two individuals with OCB may be attracted to each other, or a person with tics may find this symptom less unappealing in a potential mate.

Our findings suggest that bilineal transmission with frequent homozygosity likely explains the high density of TS observed in many families, might be an important determinant of disease severity, and may explain the failure of current attempts using high-density kindreds to localize the gene defect or defects by linkage analysis.

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A Patient with both Gilles de la Tourette syndrome and the chromosome 22q11 deletion syndrome: Is it a clue to the genetics of Gilles de la Tourette syndrome?

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Abstract

This is the first case description in the published literature of the association of definite Gilles de la Tourette syndrome (GTS) and the chromosome 22q11.2 deletion syndrome, previously referred to as CATCH-22 syndrome. *The co occurrence of GTS, 22q11DS and their behavioural/neuropsychiatric abnormalities may be due to the common endophenotypic mechanisms shared by these disorders, rather than being specific for GTS.* Research into this genomic region may lead to advancement in neurobehavioural/neuropsychiatric genetics that will help us in further explicating the broader perspective of gene-brain-behaviour inter-relationship and genetic underpinnings of various developmental psychopathologies, neuropsychiatric and behavioural disorders that are common to both GTS and 22q11DS. *Our report should warrant further genetic investigation of chromosome 22q11.2 deletion site using alternative strategies of quantitative trait loci - endophenotype based approach which would be useful for establishing the biological and molecular underpinning of OCD, ADHD and GTS.*

Keywords: Gilles de la Tourette syndrome, CATCH 22 syndrome, microdeletion syndrome, chromosome 22q11.2 deletion syndrome, *endophenotype*, genetics.

Introduction

Gilles de la Tourette syndrome (GTS) is a complex neuropsychiatric developmental disorder with high comorbidity rates with other neurobehavioural disorders and psychopathologies [1]. This includes attention deficit hyperactivity disorder (ADHD), obsessive-compulsive behaviours (OCB) or disorder (OCD), self injurious behaviour (SIB), depression, anxiety, bipolar affective disorder, personality disorder, drug abuse, rage attacks, and increased irritability and cognitive abnormalities such as executive dysfunction [2,3,4]. It

seems likely that these disorders share a common or overlapping neurobiological basis. The aetiopathogenesis of GTS spectrum phenotype is multifactorial contributed by the interaction of genetic susceptibility, epigenetic factors, environmental factors, and neurobiological systems active in the developing brain. GTS is both genetically and phenotypically heterogeneous with many areas of chromosomal loci having been identified [3, 4,5]. The interested reader is referred to updates on the genetics of GTS reviewed by Pauls [6, 7].

The chromosome 22q11.2 deletion syndrome (22q11DS) is also known as the CATCH-22 syndrome which is an acronym for Cardiac defect, Abnormal facies, Thymic hypoplasia/aplasia, and T-cell deficiency, Cleft palate, Hypoparathyroidism, and Hypocalcemia and is associated with chromosomal microdeletion in the q11 band of 22 [8]. DiGeorge syndrome (DGS), Velocardiofacial syndrome (VCFS) and Conotruncal anomaly face syndrome (CTAFS) are part of 22q11DS reflecting various outcomes of the same underlying genetic defect. About 60% of patients with 22q11DS will manifest neuropsychiatric, neurobehavioural and developmental psychopathologies [9]. The comorbid behavioural/psychiatric disorders include learning disabilities, ADHD, attention deficit without hyperactivity, early onset OCD/OCS, early onset bipolar spectrum disorders, schizophrenia and schizoaffective disorder, anxiety disorder, and oppositional defiant disorder [10,11].

Of importance is that there now appears to be considerable overlap between 22q11DS and both OCD [12] and ADHD [13]. Behavioural abnormalities associated with VCFS and ADHD have been correlated with corpus callosum morphology abnormalities [14]. Similar observations have been made in GTS [15, 16]. Further research on psychopathology of 22q11DS may provide a model of how a specific genetic defect could lead to the development of neuropsychiatric/behavioural disorders. *Both these complex neuropsychiatric genetic disorders are the result of genetic probabilism, where the phenotypic output is the product of active interaction of genotype, environmental, and epigenetic factors. The genetics and genomics of such complex behavioural and*

neuropsychiatric disorders can be studied by using multifaceted approaches such as quantitative trait loci-endophenotype approach [17, 18].

Case-report

A 28 year-old female presented to the GTS Clinic for assessment of a tic disorder. She was born one month premature at 36 weeks gestation, with cyanosis at birth, and was floppy with a birth weight 3 lbs 12oz. She required an incubator for 6 weeks. In childhood, she was found to have developmental motor delays and expressive language delays, as she only walked and talked at the age of 15 months. She had insomnia and rage attacks as a youngster. She had moderate learning disabilities and comorbid ADHD. She was also diagnosed with oppositional defiant disorder (ODD) and has some symptoms suggestive of Asperger's syndrome or autistic spectrum disorder. Later, she was also found to have unexplained persistent hypocalcaemia and attributed to as idiopathic hypoparathyroidism. As a youngster she was investigated and found to have a heart murmur, but with no obvious cardiac abnormalities. Her learning difficulties were noticed early, and she attended a special nursery school at the age of three. She received a Statement of Special Educational Needs at the age of 6 years and attended a special boarding school between the ages of 6 and 19 years. She thereafter lived with her mother and other carers, with respite with grandmother and other individuals/agencies.

Her motor tics began at the age of 2 and a half to 3 years with a shoulder shrug and arm movements and excessive blinking beginning at 4 years. Vocalisations began at the age of 4-5 years. She also developed coprolalia, copropraxia, echopraxia, suggestibility, premonitory sensations, SIB and OCB (arithmomania, rearranging), but not OCD. Her tics were suppressible (with rebound afterwards), suggestible and characteristically waxed and waned in intensity. The tics had remained much the same after the age of 18 years.

Significant family history included confirmed proven 22q11 deletion in her mother (fluorescent in situ hybridization [FISH] analysis) who also suffered from repetitive sniffing (a phonic tic), in addition to the 22q11DS multi-system anomalies (hypoparathyroidism, hypothyroidism, osteoporosis and kyphoscoliosis). The patient's maternal grandmother had kyphoscoliosis, and two deceased maternal great aunts apparently had motor tics. Her maternal grandfather was anxious and depressed all his life, necessitating ECT. Maternal great aunt was diagnosed with schizophrenia. There was also a history of autistic spectrum disorders and epilepsy in the family. These latter family members were not available for the cytogenetic study for chromosome 22q11 microdeletion as they were deceased.

Examination revealed typical dysmorphic craniofacial abnormalities including prominent epicanthic folds, up-slanting palpebral fissures and marked epicanthic folds. She was impulsive and disinhibited and took no notice of obvious social cues. She fulfilled DSM IV-TR criteria for GTS. She had a history of 27 motor tics, 17 of which were observed at interview, and 8 vocal/phonic tics, 3 of which were heard during the interview. On the Yale Global Tic Severity Scale she scored 50% (moderate) and on the Diagnostic Confidence Index, she scored 82%. She used several words as substitutes for swear word such as "janus". She had a low mood and persecutory delusions. Special investigations included fluorescent in situ hybridization (FISH) which confirmed 22q11 deletion, a borderline IQ (V= 73; P = 68) and a spot of calcification of the right globus pallidus on CT scan.

Discussion

We describe a young woman of 28 years with DSM-IV- TR GTS who also presented with another complex genetic disorder with craniofacial and neuropsychiatric phenotype of 22q11DS confirmed by fluorescent in situ hybridization (FISH). Interestingly, her mother who had chronic phonic tics also had the interstitial microdeletion of chromosome 22q11.2 and many of the associated multi-system anomalies. At present, the scientific community is at

the threshold of identifying the regions harbouring the genes for the expression of the GTS phenotype, and the association of GTS with 22q11DS may be another step in our understanding of the genetic/biologic risk factors important for the expression of these 'genetically driven' behaviours. Given that there is almost certainly genetic and clinical heterogeneity in GTS, and yet undetermined phenotypic variations, it is important to document interstitial microdeletion syndromes co-occurring with GTS, particularly when two generations of individuals have both tic/GTS spectrum disorder and the chromosome 22q11.2 microdeletion as in our case reported here. The earlier study by Gothelf did document the presence of stereotypy, and motor tics in addition to comorbid psychopathology in 22q11DS that included OCD/OCS [12], and ADHD [13].

Some of the specific genes within the 22q11.2 deletion site that are implicated to central nervous system structure or functioning include GSCL gene, the ES2 gene, the UFDIL gene, PRODH gene, and the COMT gene [11,19]. COMT gene functional polymorphism has attracted intense interest because of the association of chromosome 22q and disorders at the mind-brain interface. A functional polymorphism of this gene results in the dysregulation of the dopaminergic system that underpin various dopamine-related neuropsychiatric disorders such as schizophrenia, bipolar disorder [20, 21, 22, 23], OCD [24, 25, 26], ADHD [27] and the 22q11DS neuropsychiatric phenotype [28] COMT therefore does represent one of the excellent susceptibility or candidate genes for several neuropsychiatric, behavioural and developmental psychopathologies as exemplified by the model of 22q11DS.

We acknowledge that as both GTS and the 22q11DS are now recognised to be quite common, the association of our index patient with both could be chance. However the fact that her mother has the microdeletion and phenotypic features of 22q11DS, as well and that she also has a chronic phonic tic (GTS spectrum), we would suggest that the association is not chance. *We suggest that the co occurrence of GTS and 22q11DS as illustrated by our case report might reflect common endophenotypic mechanisms underpinning both these complex genetic disorders. Thus, endophenotype research and*

'quantitative trait loci' approach are excellent strategies that may have greater potential than the conventional multiple genetic linkage/association studies to assist in the genetic dissection of these complex behavioural/neuropsychiatric disorders.

Conclusion

We believe that ours is the first report in literature of the association of the 22q11DS with DSM-IV definite GTS. We suggest that research of 22q11DS critical deletion region and the genes contained within may well be regions of interest for future genetic dissection using cytogenetic studies, genetic linkage studies, *and quantitative trait loci - endophenotype based approach.* This would be useful for establishing the biological and molecular underpinning of OCD, ADHD and GTS. Such studies will help us in further explicating the broader relationship between gene-brain and behaviour, the domain of neurobehavioural-neuropsychiatric genetics.

Human behaviour results from the interactions between complex genetic and epigenetic factors, cellular, anatomical and functional networks with environmental influences. Hence genetic approaches to dissect the gene-behavioural/psychiatric trait equation are challenging. Forward genetic approaches, reverse genetic approaches, quantitative trait loci (QTL) mapping and mouse models represent current genetic approaches for investigating the genetic basis of behaviour.

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TOURETTE SYNDROME ASSOCIATION INTERNATIONAL CONSORTIUM FOR GENETICS

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A Complete Genome Screen in Sib Pairs Affected by Gilles de la Tourette Syndrome

The Tourette Syndrome Association International Consortium for Genetics*

Summary

Gilles de la Tourette syndrome is a neuropsychiatric disorder characterized by waxing and waning multiple motor and phonic tics with a complex mode of inheritance. Previous attempts, which used large multigenerational families to localize susceptibility loci, have been unsuccessful. In this report, the results of the first systematic genome scan, using 76 affected-sib-pair families with a total of 110 sib pairs, are summarized. While no results reached acceptable statistical significance, the multipoint maximum-likelihood scores (MLS) for two regions (4q and 8p) were suggestive (MLS > 2.0). Four additional genomic regions also gave multipoint MLS scores between 1.0 and 2.0.

Introduction

Gilles de la Tourette syndrome (GTS) (MIM 137580) is a chronic neuropsychiatric disorder with onset in childhood. It is characterized by multiple, fluctuating motor and vocal tics of variable severity. Prevalence estimates are higher in males than in females, and range from 1/20,000 to 1/2,000, depending on the age group studied and on the diagnostic inclusion criteria (Apter et al. 1993). More recent studies in school-age populations suggest that the prevalence may be somewhat higher (e.g., 1% in males) (Robertson and Stern 1998). Support for a genetic component in the etiology of GTS comes from several lines of evidence, including twin studies (Price et al. 1985; Hyde et al. 1992) and family studies (Pauls et al. 1991). Family studies also provide support for a common genetic basis for GTS, chronic tic disorder (CT), and obsessive-compulsive disorder (OCD) (Pauls et al. 1986). In addition, a relationship with other psychiatric disorders has been suggested (Comings and Comings 1985). While the relationship between GTS and other conditions has been somewhat controversial, the recurrence risk for GTS among first-degree relatives appears to be quite consistent among studies. Pauls et al. (1991) reported a recurrence risk for GTS of 7/61 (11.5%) in brothers and 4/83 (4.8%) in sisters of probands. Walkup et al. (1996) and Hebebrand et al. (1997) observed recurrence risks that were not significantly different from those reported by Pauls and colleagues.

Segregation analysis generally supports the presence of a major locus, but the characteristics for this predisposing gene vary. Pauls et al. (1990) reported that the pattern of transmission was consistent with autosomal dominant inheritance with incomplete penetrance, whereas Hasstedt and colleagues (1995) found the pattern in large multigenerational families in which there was bilineal transmission to be consistent with a genetic model in which the penetrance of the heterozygote was between the penetrance of the two homozygotes. Walkup et al. (1996) reported a similar solution but with a significant multifactorial background. Several studies have reported the frequent occurrence of bilineal families, in which either GTS, CT, or OCD occurs in both parents of a proband or in one of the parents' first-degree relatives (McMahon et al. 1992; Kurlan et al. 1994; McMahon et al. 1996; Walkup et al. 1996).

Attempts to localize the responsible gene(s) have not yielded consistent results thus far. Linkage analysis of data from a series of multiply affected families resulted in the exclusion of ~80% of the genome (for review, see Barr and Sandor 1998). These analyses were completed assuming a dominant mode of inheritance and locus homogeneity for GTS and spectrum disorders. This data set included several very large families, which, on their own, could yield significant evidence for linkage (Heutink et al. 1995). It is possible that incorrect specification of genetic parameters (e.g., penetrance and gene frequency) of the GTS susceptibility gene could have led to false exclusion of one or more relevant genomic regions.

More recently a genome scan was completed on a series of multigenerational families (Barr et al. [1999]) that included two families that had also been examined

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by Heutink et al. (1995). This genome scan used more informative markers that were evenly spaced throughout the genome. The data were analyzed using both parametric (autosomal dominant with reduced penetrance) and nonparametric methods. LOD scores >1.0, calculated with the parametric method, were observed for 24 of the markers in at least one of the families. No LOD scores >2.0 were observed. When the Affected Pedigree Method (APM; Weeks and Lange 1988) modified by Ward (1993) was used, eight markers were observed with a $P < .00005$ in at least one of the families. APM has been shown to be more likely to give false positive results (Field and Kaplan 1998), so these results should be interpreted with caution.

Other approaches have also been employed in the search for GTS-susceptibility loci. Several families have been reported with apparent cosegregation of balanced translocations with GTS (for a review, see Robertson and Boardman 1996), but these candidate regions have not been supported by positive linkage findings in other families (Heutink et al. 1990). In addition, a number of association studies have been published examining a variety of candidate genes in GTS patients and controls (Comings et al. 1991; Comings et al. 1993; Nöthen et al. 1994*a*, 1994*b*; Grice et al. 1996). Finally, Simonic and colleagues (1998) performed a genomewide search (with genetic markers at an average spacing of 3 cM) using a case-control strategy in a sample of unrelated patients with GTS from an Afrikaner population. These investigators reported positive associations between GTS and markers on chromosomes 11, 14, 20, and 21 that were highly significant before correction for multiple testing. While these are potentially interesting leads, association studies should be interpreted with caution. It has been demonstrated that false positive findings can occur when controls are not perfectly matched with respect to their genetic background (Kidd 1993). It should be noted that none of the positive reports from association studies have been replicated.

Analysis of affected sib pairs has been proposed as an alternative to parametric linkage analysis and association studies. The sib-pair approach is suited for diseases with an unclear mode of inheritance and has been used successfully in studies of other complex disorders. Thus, given the lack of conclusive results from more traditional linkage and association studies, an affected-sib-pair study appears to be an appropriate strategy to identify chromosomal regions linked to GTS.

The overall contribution of genetic factors to disease susceptibility can be measured conveniently by comparing the risks for various types of relatives of probands with the risk of the disease in the general population (Risch 1990*a*). When calculated in this way, the relative risk for sibs of GTS probands is sufficiently high ($\lambda_{sib} > 10.0$) to suggest that affected sib-pair analysis is a viable alternative to traditional parametric linkage analysis for GTS. In this report, the results of a complete genome screen using 370 highly polymorphic DNA markers is reported. The sample for this genome scan consisted of 76 affected-sib-pair families with at least one parent available for clinical evaluation and genotyping. These 76 families yielded a total of 110 sib pairs. Correcting for nonindependence resulted in a sample that was equivalent to 91 independent pairs.

Material and Methods

Sample

All affected-sib-pair families consisted of at least two sibs affected with GTS. Furthermore, in the ascertainment of families, if both parents were affected with GTS or if one parent had GTS, CT, OCD, and/or subclinical OCD and the other parent also received a diagnosis of CT, OCD, and/or subclinical OCD the family was not included in the analyses reported here. All diagnoses were made using DSM-III-R criteria. The criteria for subclinical OCD were the same as those used to make a diagnosis of OCD, with the exception that the individual did not perform the compulsions or obsessions for at least an hour, did not experience them as egodystonic, or did not report any impairment. These were the same criteria used in the family study of OCD reported by Pauls et al. (1995). The final sample included in the genome scan consisted of 76 families with at least two sibs affected with GTS. Both parents were included in the tests for 72 families. Among these 72 families, there were 19 in which neither parent was affected with GTS, tics, or OCD; 32 in which the father was affected and the mother was unaffected; and 21 in which the mother was affected and the father was unaffected. In the remaining four families, only one parent was tested; in every case that parent was unaffected. Of the 76 families, 64 had only two children who were affected, 10 had three affected sibs, and the remaining 2 had four and five affected sibs, respectively.

Phenotypic Evaluation

When a family entered the study, information concerning both affected siblings and their parents was collected in a two-stage process. The initial stage consisted of the collection of information concerning symptoms associated with GTS and OCD using a self-and-family report based on the tic inventory and ordinal severity scales of the Yale Global Tic Severity Scale (Leckman et al. 1989) and the symptom checklist and ordinal scales of the Yale-Brown Obsessive-Compulsive Scale (Goodman et al. 1989). Earlier versions of these instruments have been used in prior studies of individuals with GTS and OCD and have been shown to have a high level of

agreement with expert clinician ratings of tic and obsessive-compulsive symptom severity (Leckman et al. 1993, 1994, 1997). In a second stage, the validity of these symptom ratings were reviewed by an experienced clinician to insure their accuracy and validity. These instruments are currently being used in family studies of both GTS and OCD. Earlier versions have been shown to be both valid, when compared to clinician diagnoses (the rates of agreement between interview derived diagnoses and clinical diagnoses were .98 for GTS and .97 for OCD), and reliable, for the diagnoses of GTS (κ = 1.00) and OCD (κ = .97) (Pauls et al. 1995). For the assessment of other psychopathology, the Kiddie Schedule for Affective Disorders and Schizophrenia (Chambers et al. 1985; Kaufman et al. 1995) was used for children aged <18 years and the Structured Clinical Interview for DSM-III-R (Spitzer et al. 1992) was used for adults. Both interviews have established reliability. For this report, only sibs with a diagnosis of GTS were considered to be affected. Future analyses will include information about chronic tics, OCD, attention deficit hyperactivity disorder, and other comorbid conditions.

Best-Estimate Diagnoses

All diagnoses were made using the best-estimate approach (Leckman et al. 1982). The best-estimate procedure used in the present study followed a standard protocol. Before the initial diagnostic estimate was made, separate files for each individual were prepared. These files contained all available information about the individual, including the completed interview packet and medical records, when available. All of this information was reviewed by three clinicians (B. v.d.W., Rotterdam site; W. M., Utah site; R. A. K., Yale site) who independently made diagnostic assessments. All three diagnosticians were blind to the prior diagnosis of the individual and to his/her relationship to the proband. Each interview was evaluated by two raters. The best estimates of the two diagnosticians were then compared. The rate of agreement between any two diagnosticians was very high (κ = .97) for the diagnosis of GTS. When there was disagreement between the two raters, the individual files were reviewed by the third diagnostician and a final consensus diagnosis was assigned. These consensus diagnoses were then compared with the diagnosis assigned by the clinician at the site where the family was recruited. If there were differences, the clinical materials were reviewed via a conference call and consensus was reached if possible. If there was still disagreement, more data were requested to help resolve the differences. If there was still disagreement, the family was removed from the sample. For the current report, only diagnoses of GTS, chronic tics, OCD, and ADHD were made.

DNA Markers

Short tandem-repeat polymorphisms (STRPs) from Marshfield Screening Set 8 (Yuan et al. 1997) were genotyped in Marshfield. The markers chosen were at a 10-cM average density.

Data Analysis

Allele frequencies for the genetic markers were established by gene counting in genotyped parents. Tests for linkage were done under the direction of LS (Rotterdam site) and were performed following the maximum-likelihood–score (MLS) approach (Risch 1990*b*). In general, the MLS does not depend on marker allele frequencies when the parents' genotypes are available, which was the case for almost all families in the present study. For each pair of affected sibs, the identity-by-descent (IBD) distribution was estimated by single-point and multipoint analysis. In the single-point analysis, the IBD distribution was estimated given the marker genotypes for each marker individually. In the multipoint analysis, MLS values were computed for >4,000 different locations relative to the markers (average step size <1 cM).

For all calculations the MAPMAKER-SIBS program (Kruglyak and Lander 1995) was used. In the estimation of the IBD distribution, this program only considers IBD vectors that are biologically consistent, lying within the "possible triangle" as described by Holmans (1993). All MLS calculations allowed for dominance variance. In addition to these MLS calculations, the information content of the marker map was evaluated, and likelihoods were calculated for a fixed value of λ_s of 2.0 under the assumption of no dominance variance, which leads to an IBD vector of (z_0, z_1, z_2) = (.125, .5, .375). A comparison of these likelihoods with the likelihoods obtained for the Mendelian expectation (z_0, z_1, z_2) = (.25, .5, .25) yields exclusion LOD scores for a GTS-predisposing gene with a λ_{sib} = 2 (Kruglyak and Lander 1995).

For families with more than two affected siblings, all possible pairs were evaluated, and the results were weighted by a factor equal to $2/n$, where n indicates the number of affected children in the sibship.

Results

As noted above, the 76 families yielded a total of 110 sib pairs. Weighting by a factor of $2/n$ resulted in a sample equivalent to 91 independent sib pairs. The power of this sample is sufficient to detect linkage when λ_{sib} is $\geq$4. When equations published by Risch and Merikangas (1996) are used, this sample of 91 sib pairs has power of 59% to map a given locus with λ_{sib} = 2, and 99.9% when λ_{sib} = 4.

The panel of markers genotyped included 370 DNA

markers with an average spacing of 9.1 cM in the male meiotic map. The observed average heterozygosity of this set of markers was .77. Single-point analyses with dominance variance yielded MLS values of 2.38 and 2.09, respectively, for markers D4S1625 and D8S1106. Twelve additional markers (D1S1728, D4S403, D4S2623, D4S1644, D6S1053, D8S1130, D8S1145, D8S136, D10S1213, D11S912, D14S592, and D17S1298) yielded MLS values between 1.0 and 2.0. Two of these markers (D4S2623 and D4S1644) are located in the vicinity of D4S1625, and three (D8S1130, D8S1145, and D8S136) are in the vicinity of D8S1106. Graphs of multipoint MLS values are shown infigure 1. Two regions show suggestive MLS values ≥ 2.0. On chromosome 4, MLS values exceeded 2.0 over a region of ~12 cM, including markers D4S1644 and D4S1625, with a peak MLS value of 2.3 at ~3 cM telomeric of D4S1625. The estimated IBD values at this location were $(z_0, z_1, z_2) = (.185, .370, .445)$. On chromosome 8, MLS values reached 2.0 in two adjacent intervals, bounded by markers D8S1106, D8S1145, and D8S136, respectively. Here the estimated IBD values were $(z_0, z_1, z_2) = (.146, .442, .412)$. As can be seen in figure 1, four additional regions show multipoint MLS values >1.0. These regions are on chromosomes 1, 10, 13, and 19. Finally, summing over all regions for which the LOD score was <-2.0 suggests that ~35% of the genome has been excluded, assuming $\lambda_{sib} = 2$.

Discussion

When judged purely on the basis of twin and family studies (Price et al. 1985; Pauls et al. 1991; Hyde et al. 1992; Hebebrand et al. 1997), the evidence for a substantial genetic contribution to the etiology of GTS is convincing. Even when the highest estimates for the population prevalence of GTS are considered, the risk for first-degree relatives of probands is increased by at least a factor of 10. Thus, it is surprising that genetic linkage studies have so far been unsuccessful. It is possible that such an increased risk in close relatives of probands could be due to shared environmental factors. If such factors exist, the outlook for their detection is much better than that for the detection of similar factors in some late-onset diseases, such as multiple sclerosis, because the time window, between birth and onset of disease, for such factors to act would be quite small. That no clear risk factors have yet been identified in the familial shared environment is in agreement with the results of segregation analysis. To date, all segregation analyses for GTS consistently yield evidence that the risk for GTS is transmitted in a Mendelian fashion and that there are genes of major effect that contribute to its manifestation (Pauls et al. 1990; Hasstedt et al. 1995; Walkup et al. 1996).

The results of segregation analysis are also consistent with the observed high relative risks for close relatives of probands. Given these high relative risks, many investigators believed that it should be relatively simple to map the gene(s) responsible for GTS in one or a few large multiply affected families. These efforts have not resulted in convincing linkage findings, even when the existing large families were considered separately (Heutink et al. 1995; Barr et al. [1999]). It is conceivable that extensive locus heterogeneity might preclude one from detecting linkage in a series of unrelated families. In that case, however, one would expect to find suggestions for linkage in the existing large families, albeit for each family in a different location. It is noteworthy that Barr and colleagues (1999) did observe suggestive evidence for regions on chromosomes 5 and 19p.

One potential pitfall in the study of large families has been discussed (Pauls 1993): the very unpredictable and highly biased ascertainment procedure that leads to the identification of large multiply affected families may lead to identification of families segregating for more than a single disease gene, even when those disease genes are rare in the general population. A general problem in the early statistical analyses of linkage in family data was the choice of genetic model parameters—most importantly, the gene frequency and the penetrances for the different genotypes. Serious misspecification of these parameters may lead to false exclusion of the region of interest (Heutink et al. 1995). Furthermore, it is not appropriate to rely completely on the results of segregation analysis for this purpose. When locus heterogeneity exists, and some loci act recessively while others act dominantly, the joint segregation analysis of these families may yield intermediate results that are inadequate for analysis of linkage in each individual family. The models chosen in earlier mapping efforts were broadly in agreement with the results of segregation analysis and family studies: dominant, with a low gene frequency and a relatively low phenocopy frequency, leading to a high recurrence risk in families.

Given that the early mapping studies were inconclusive, a new series of families with sib pairs affected with GTS were ascertained for a complete genome scan. It should be emphasized that all affected sibs met diagnostic criteria for GTS. That is, in contrast to linkage studies in large families, no relatives with CT or OCD were considered as affected in the sib pairs. Furthermore, all linkage analyses were done using nonparametric methods.

Two areas, one on chromosome 4q and another on chromosome 8p, are suggestive of linkage. When viewed from a pessimistic standpoint, the results are disappointing, in that no single area of the genome showed significantly increased IBD sharing in the affected siblings. It is possible that the increased allele sharing in either

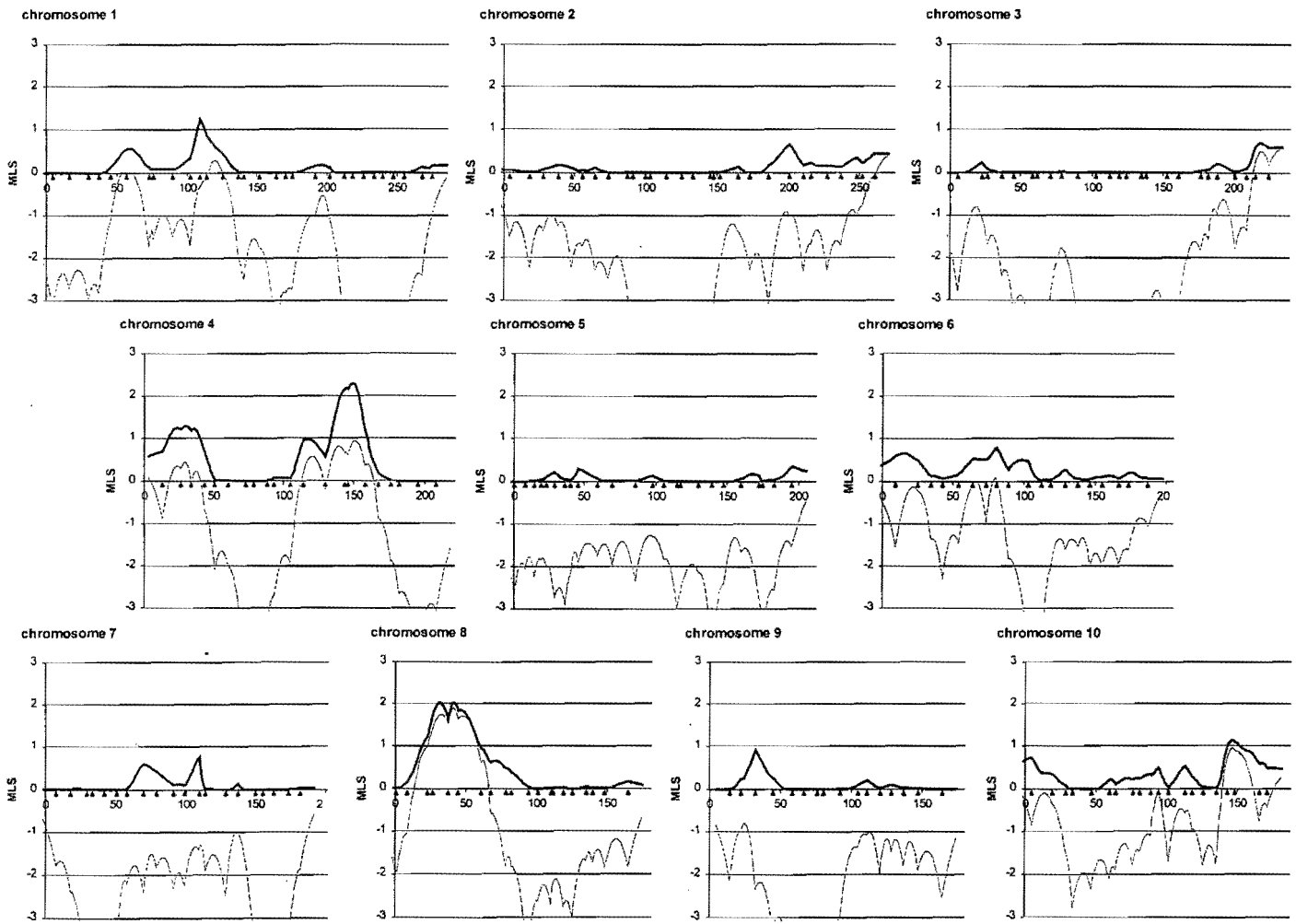

Figure 1 Graphs of multipoint maximum-likelihood scores for all chromosomes. The bold top line represents evidence for linkage; the bottom line represents exclusion. The triangles represent the location of the genotyped markers. All exclusions are based on an assumption that $\lambda = 2.0$s.

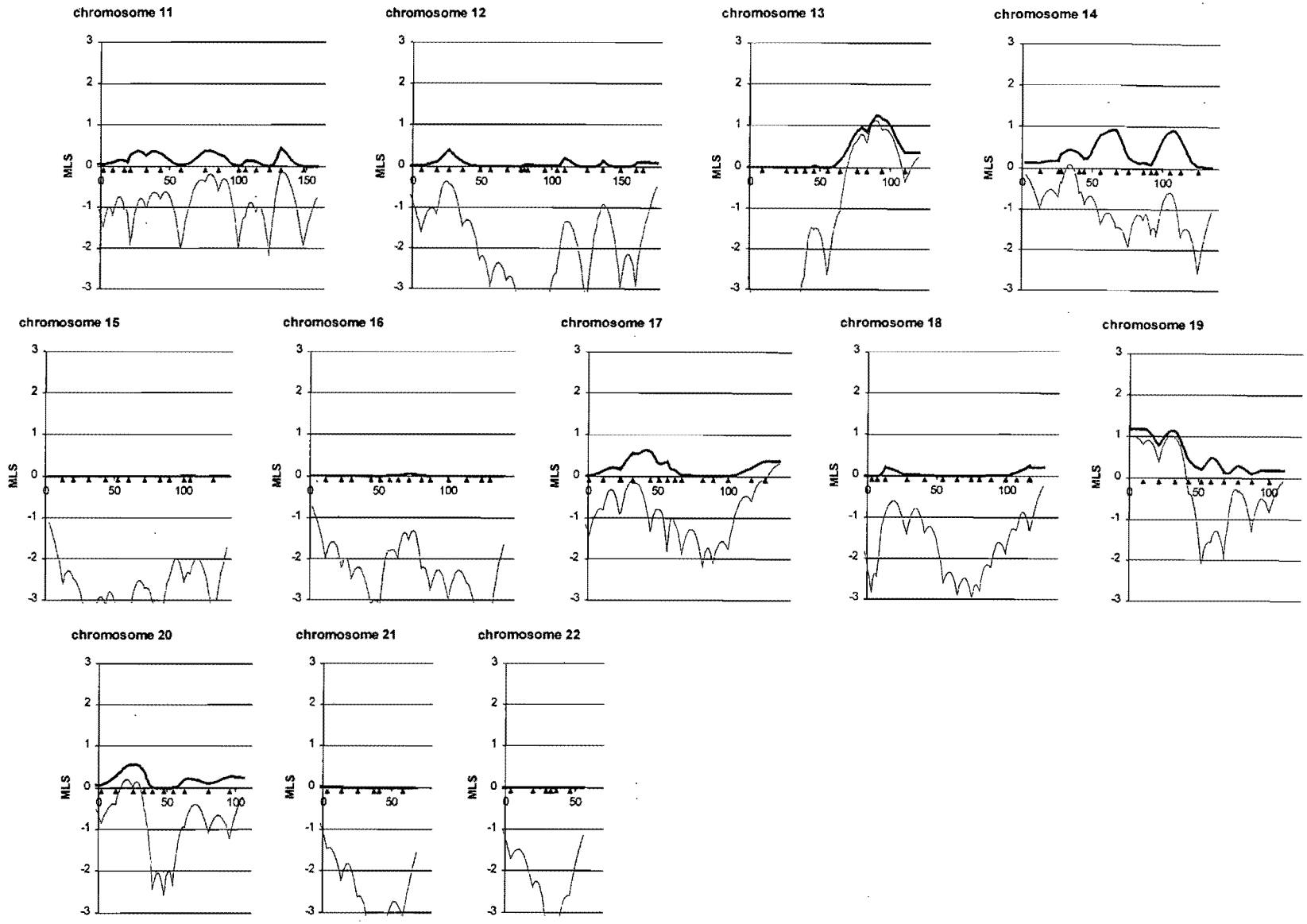

Figure 1 *(Continued)*

of these regions is only due to chance. On the other hand, these peaks in the MLS curve may reveal the true location of genetic risk factors for GTS. Certainly, these results suggest that additional typings are needed in these areas, as well as in the other regions of the genome in which there are suggestive MLS scores (i.e., $1.0 < MLS < 2.0$). At present, additional genotyping is under way in the available multigenerational families and will be completed in new affected-sib-pair families that are currently being evaluated. It should be recognized that if these peaks represent real genetic risk factors, it is likely that they only convey mildly increased risks.

It is clear that for further analysis of these regions in the available extended families, nonparametric methods should be employed. Given the relative risks of these loci, it is unlikely that the appropriate genetic mechanism could be adequately modeled. In this respect, it is important to observe that the sharing pattern observed for 4q is most consistent with a recessive contribution in this region: the increased sharing is exclusively based on an excess of affected sib pairs sharing two parental alleles. On the basis of the ascertainment procedures followed in the collection of the existing series of extended families, it is unlikely that a recessively acting gene would play a prominent role in those families, although in several of the large families there is evidence for bilineal inheritance of milder forms of the syndrome (i.e., CT and/or OCD). At the present time, there is no way of knowing whether the locus on 4q contributes to the expression of CT and/or OCD. Therefore, further analysis of this region should be mostly based on findings in additional sib pairs affected with GTS.

It is important to note that the sharing pattern for 8p is most consistent with a dominant contribution in this region. That is, the increased sharing comes from sib pairs sharing just one parental allele. This is also generally true for the other four genomic regions that have multipoint MLS scores greater than 1.0. Thus, the existing large families should be helpful in understanding more completely the genetic mechanisms in these areas. Nevertheless, more affected sib pairs are needed to follow up these results as well.

It is somewhat disappointing that none of the several chromosomal regions (e.g., 3 [3p21.3], 8 [8q21.4], 9 [9pter], and 18 [18q22.3]) in which there were cytogenetic abnormalities cosegregating with GTS and related conditions showed any strong evidence for linkage in these families. Furthermore, none of the regions in which associations had been reported with candidate genes are supported by these results (e.g., DRD2 [11q22] and DRD4 [11p15]). It is still possible that there are genetic loci in the regions identified by the cytogenetic abnormalities that confer some risk for GTS and related conditions and that the reason that they were not seen in the current study is that only GTS was examined. If that is the case, then it could be concluded that the cytogenetic abnormalities are more likely to be associated with the related conditions and that the GTS seen in these families could be etiologically different than that observed in these sib-pair families.

It is noteworthy that one region that has been suggested from a genome scan of multigenerational families (chromosome 19p; Barr et al. [1999]) is also positive in the current study. Clearly, this and the other positive regions need to be explored more thoroughly to determine whether these findings represent true linkages.

In sum, the results of the first systematic genomewide scan for GTS suggest that there are several genes of moderate effect that increase susceptibility to GTS. Further work is needed to confirm and extend these findings. While it is possible that the current results are false positives, they represent the best available data as to the location of susceptibility loci for this complex neuropsychiatric disorder of children and adults.

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Electronic-Database Information

A URL for data in this article is as follows:

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Genomewide Scan of Hoarding in Sib Pairs in Which Both Sibs Have Gilles de la Tourette Syndrome

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A genome scan of the hoarding phenotype (a component of obsessive-compulsive disorder) was conducted on 77 sib pairs collected by the Tourette Syndrome Association International Consortium for Genetics (TSAICG). All sib pairs were concordant for a diagnosis of Gilles de la Tourette syndrome (GTS). However, the analyses reported here were conducted for hoarding as both a dichotomous trait and a quantitative trait. Not all sib pairs in the sample were concordant for hoarding. Standard linkage analyses were performed using GENEHUNTER and Haseman-Elston methods. In addition, novel analyses with a recursive-partitioning technique were employed. Significant allele sharing was observed for both the dichotomous and the quantitative hoarding phenotypes for markers at 4q34-35 ($P = .0007$), by use of GENEHUNTER, and at 5q35.2-35.3 ($P = .000002$) and 17q25 ($P = .00002$), by use of the revisited Haseman-Elston method. The 4q site is in proximity to D4S1625, which was identified by the TSAICG as a region linked to the GTS phenotype. The recursive-partitioning technique examined multiple markers simultaneously. Results suggest joint effects of specific loci on 5q and 4q, with an overall P value of .000003. Although P values were not adjusted for multiple comparison, nearly all were much smaller than the customary significance level of .0001 for genomewide scans.

Introduction

In his original description of Gilles de la Tourette syndrome (GTS [MIM *137580]) in 1885, Gilles de la Tourette noted the presence of obsessive-compulsive symptoms in several of the patients he studied. Subsequent studies have shown prevalences of obsessive-compulsive symptoms of 11%–80% among individuals with GTS (King et al. 1998). This wide range in prevalence likely reflects differences not only in the sample composition but also in the assessment instrument and criteria used. Although obsessive and compulsive features are common in individuals with GTS, the proportion whose symptoms are sufficiently severe to warrant a diagnosis of obsessive-compulsive disorder (OCD [MIM 164230]) is considerably smaller, with only ~30% of adults with GTS meeting the full criteria for obsessive-compulsive disorder. These elevated prevalences of obsessive-compulsive symptoms are found not only in clinical samples composed of patients with GTS but also in nonreferred individuals with tics who were identified in community samples (Apter et al. 1993), as well as in first-degree relatives of individuals with tics (Pauls et al. 1991). The obsessions and compulsions found in individuals with GTS cover a broad range in terms of content, intensity, persistence, impairment, degree of perceived ego-syntonicity, and relationship to the individual's tic symptoms. A growing number of studies examining symptom type, natural history, sex ratio, family-genetic data, neurobiological correlates, and treatment response lend increasing support to the hypothesis that tic-related obsessive-compulsive disorder constitutes a distinctive obsessive-compulsive disorder phenotype (Leckman et al. 2000*a*).

OCD, considered separately from GTS, is a chronic disability affecting 1%–3% of the general population (Horwath and Weissman 2000). Patients with OCD describe the sudden intrusion into consciousness of unwanted worries or unpleasant images, as well as repeated urges to perform seemingly senseless acts. Standard nomenclatures designate OCD as a unitary nosological entity (American Psychiatric Association 1994). Although this parsimony has a certain appeal, it is misleading. The symptoms used to define OCD are diverse and include various intrusive thoughts, pre-

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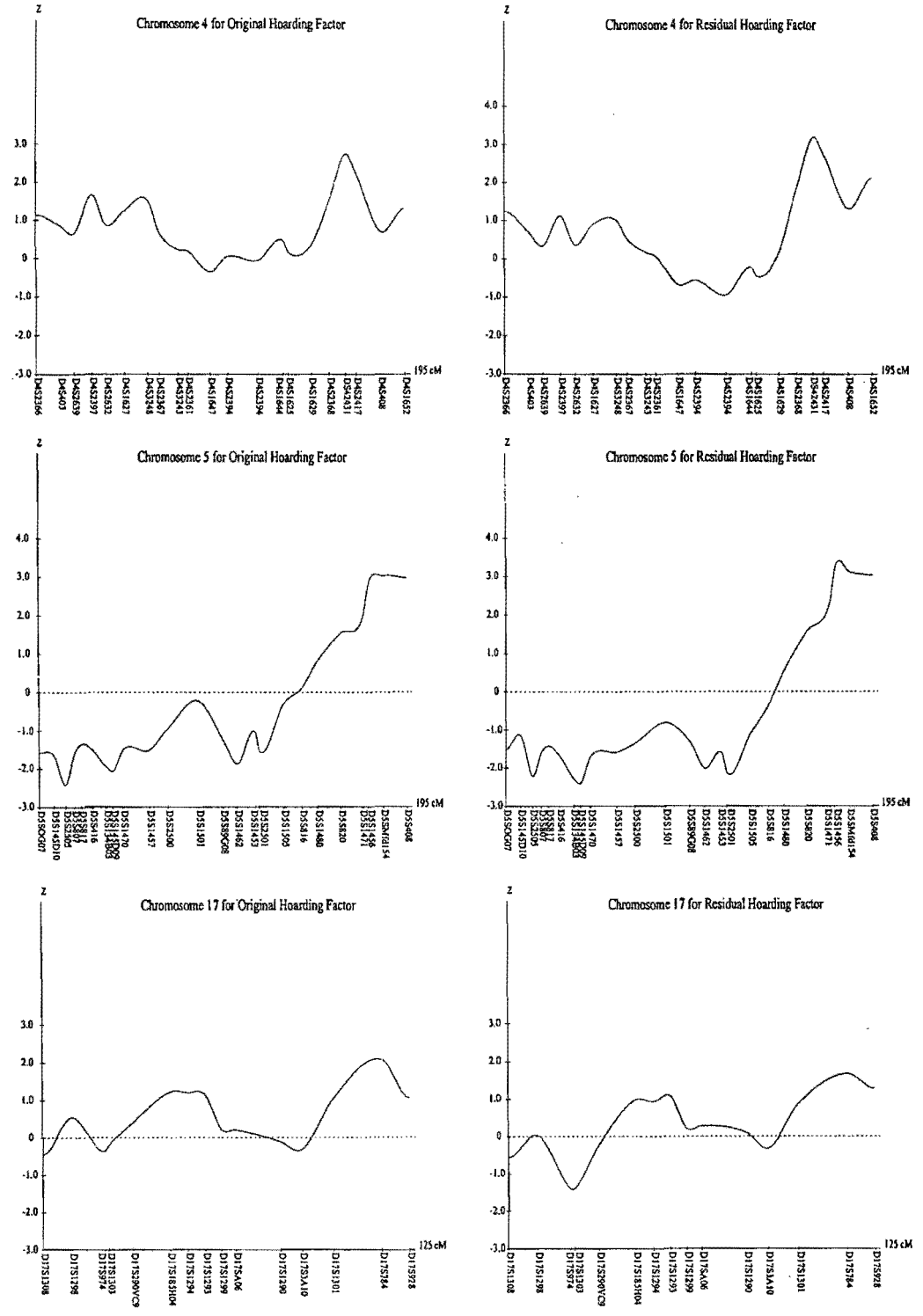

Figure 1 Nonparametric Z scores produced by GENEHUNTER for original and residual hoarding factor scores on chromosomes 4q (*top*), 5q (*middle*), and 17q (*bottom*).

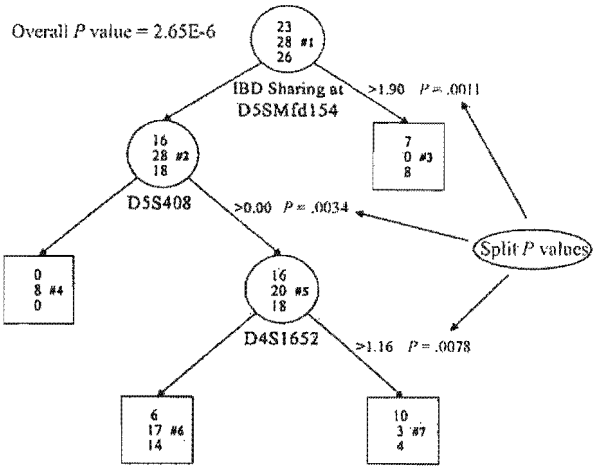

Figure 2 Tree-based genome scan for linkage to hoarding. Nodes are labeled "1"–"7." Inside each node, the numbers of sib pairs in which both members are affected (*top number*), only one member is affected (*middle number*), and neither member is affected (*bottom number*) are given. Thus, the three numbers indicate, from top to bottom, the numbers of both affected, discordant, and both unaffected pairs in any given node. IBD sharing is used to split nodes. Selected markers and cut-off values are below and to the bottom right of the node, respectively. *P* values calculated using Fisher's exact test are presented for each node split and for the distribution among all terminal nodes (*boxes*).

occupations, rituals, and compulsions, many of which are found at lower frequencies in unaffected populations.

Although the subtyping of patients with OCD on the basis of specific proband characteristics (e.g., age at onset or absence of motor or vocal tics) may lead to increased biological homogeneity, other quantitative approaches may prove to be of greater value in the identification of the relevant genetic risk factors. Factor analyses of patients with OCD have identified several obsessive-compulsive symptom dimensions (Baer 1994; Leckman et al. 1997; Mataix-Cols et al. 1999; Summerfeldt et al. 1999), including the following factors:

1. Obsessions about harm, sex, religion, and the body, as well as checking compulsions;
2. Obsessions about a need for symmetry or exactness, repeating rituals, counting compulsions, and ordering/arranging compulsions;
3. Contamination obsessions and cleaning/washing compulsions;
4. Hoarding obsessions and compulsions.

Data supporting the validity of these obsessive-compulsive symptom dimensions have been provided by studies of psychiatric comorbidity, functional brain imaging, treatment response, and studies of normal development (Leckman et al. 2001). Hoarding symptoms, in particular, appear to be associated with increased psychiatric comorbidity (Mataix-Cols et al. 1999; Frost et al. 2000), as well as poor response to standard pharmacotherapies and cognitive-behavioral treatments (Black et al. 1998; Mataix-Cols et al. 1999). Although these studies suggest that the presence of hoarding symptoms is useful for prognosis, there has been little examination of the familial or genetic factors that may contribute to their expression.

The mode of inheritance of OCD has been investigated by means of segregation analysis in five studies. Evidence of a gene of major effect was found in each of the studies that classified relatives according to the presence or absence of OCD as a binary outcome (Nicolini et al. 1991; Cavallini et al. 1999; Nestadt et al. 2000*a*). For example, Nestadt et al. (2000*a*) conducted complex segregation analyses of OCD in 153 families (80 case and 73 control families) that were ascertained in the Johns Hopkins OCD Family Study, and they reported strong evidence for a major autosomal dominant gene with significant sex effects.

Alsobrook et al. (1999) and J. F. Leckman, D. L. Pauls, H. Zhang, M. C. Rosario-Campos, L. Katsovich, K. K. Kidd, A. J. Pakstis, J. P. Alsobrook, M. M. Robertson, W. M. McMahon, J. T. Walkup, B. J. M. van de Wetering, R. A. King, D. J. Cohen, and the Tourette Syndrome Association International Consortium for Genetics (TSAICG) (unpublished data) have reported similar results by use of symptom-based factor scores. Thus far, only the aforementioned study by Leckman et al., which was undertaken as part of the TSAICG, has specifically focused on the role that genetic factors play in the transmission and expression of hoarding symptoms. They found evidence in support of a recessive mode of transmission for the hoarding symptom dimension in families with two affected siblings with GTS (J. F. Leckman, D. L. Pauls, H. Zhang, M. C. Rosario-Campos, L. Katsovich, K. K. Kidd, A. J. Pakstis, J. P. Alsobrook, M. M. Robertson, W. M. McMahon, J. T. Walkup, B. J. M. van de Wetering, R. A. King, D. J. Cohen, and the TSAICG, unpublished data). The same segregation analyses also indicated that the transmission of factors 1 and 2 was consistent with dominant major gene effects, whereas the pattern of transmission for factor 3 was consistent with recessive inheritance. Unlike factor 4 (i.e., the hoarding factor), the other three factors comprise more than one symptom. Thus, the understanding of the linkage analysis is more challenging, and the results will be published separately. The goal of the present study was to conduct a genome analysis focused on hoarding symptoms, treated as both a quantitative trait and a dichotomous variable.

Table 1

Results of Genomewide Scan Significance of the Hoarding Factor Score by Use of a Nonparametric Likelihood Method and Haseman-Elston Methods

Region and Nearest Marker	*P* Value in Analysis of: Original Hoarding Factor			Residual Hoarding Factor		
	SIBPAIR[a]	SIBPAL2[b]	GH-NPL[c]	SIBPAIR[a]	SIBPAL2[b]	GH-NPL[c]
4q34-35:						
D4S2431	.092	.006	.003	.171	.011	7E-4
D4S2417	.111	.005	.012	.153	.005	.003
D4S408	.264	.034	.232	.216	.040	.092
D4S1652	.012	.031	.090	.005	.047	.016
5q35.2-35.3:						
D5S1471	.036	.031	.023	.022	.029	.009
D5S1456	.003	.005	.002	.002	.004	6E-4
D5SMfd154	2E-4	.001	.001	3E-4	.002	9E-4
D5S408	2E-5	2E-6	.001	6E-5	3E-6	.001
17q25:						
D17S1301	.005	1E-4	.147	.007	2E-4	.189
D17S784	3E-4	2E-5	.019	8E-4	6E-5	.047

[a] Traditional Haseman-Elston method by use of S.A.G.E. (version 6 beta).
[b] Revisited Haseman-Elston method by use of S.A.G.E. (version 6 beta).
[c] Nonparametric-likelihood method in GENEHUNTER.

Subjects, Material, and Methods

Sample

All families include at least two siblings with GTS. In the original ascertainment, families were excluded if both parents were affected with GTS or if one parent had GTS, CT (chronic tics), OCD, and/or subclinical OCD and the other parent also had CT, OCD, and/or subclinical OCD. All diagnoses were made by use of *Diagnostic and Statistical Manual*–III-R criteria (American Psychiatric Association 1994). The criteria for subclinical OCD were the same as those used to make a diagnosis of OCD, except that the individual did not perform the compulsions or obsessions for at least an hour, did not experience them as ego-dystonic, or did not report any impairment. These were the same criteria used in the family study of OCD reported by Pauls et al. (1995). The final sample included in the genome scan consisted of 51 families with a total of 77 sib pairs and 223 individuals (including parents). Of the 77 pairs, 26 are concordant for hoarding, 28 are discordant for hoarding, and 23 are concordant for being unaffected with hoarding. This is a subset of the families that were included in the original genome scan of GTS reported by the TSAICG (1999), and the rest of the families are no longer available to the TSAICG. J. F. Leckman, D. L. Pauls, H. Zhang, M. C. Rosario-Campos, L. Katsovich, K. K. Kidd, A. J. Pakstis, J. P. Alsobrook, M. M. Robertson, W. M. McMahon, J. T. Walkup, B. J. M. van de Wetering, R. A. King, D. J. Cohen, and the TSAICG (unpublished data) present detailed demographic and clinical information for sample that we studied.

Phenotypic Evaluation

When a family entered the study, information concerning both affected siblings and their parents was collected in a two-stage process. The initial stage consisted of (1) the collection of information concerning symptoms associated with GTS, (2) diagnosis of OCD through an interview developed specifically for the TSAICG (i.e., a self-and-family report [TSAICG 1999] based on the tic inventory and ordinal severity scales

Table 2

Alleles Shared by Sib Pairs for Selected Markers

Sib Pair	Shared Alleles, by Type[a]: 1	2	3	4	5	6	7	9
Node 3:[b]								
AA	1	1	0	0		8	3	1
AU	0	0	0	0		0	0	0
UU	1	2	3	6		3	1	0
Node 5:[c]								
AA	4	0	0	12	2	0	0	
AU	3	1	1	9	4	0	1	
UU	4	0	2	8	6	2	0	
Node 7:[d]								
AA	4	8	7	0	1			
AU	1	2	1	2	0			
UU	4	3	1	0	0			

Note.—Selected markers are shown in figure 2. AA = both affected; AU = discordant; UU = both unaffected.
[a] Allele types at the selected markers were numbered consecutively from 1.
[b] IBD at D5SMfd154 > 1.9.
[c] IBD at D5SMfd154 ≤ 1.9, and IBD at D5S408 > 0.
[d] IBD at D5SMfd154 ≤ 1.9, IBD at D5S408 > 0, and IBD at D4S1652 > 1.16.

of the Yale global tic severity scale [Leckman et al. 1989]), and (3) the review of the symptom checklist and ordinal scales of the Yale-Brown obsessive-compulsive scale (Y-BOCS) (Goodman et al. 1989). Earlier versions have been shown to be both valid ($\kappa = 0.98$ for GTS; $\kappa = 0.97$ for OCD) and reliable ($\kappa = 1.00$ for GTS; $\kappa = 0.97$ for OCD) (Leckman et al. 1993, 1994, 1997; Pauls et al. 1995). In the second stage, these symptom ratings were reviewed by an experienced clinician during a face-to-face interview with the informant, to insure their accuracy and validity. These instruments are currently being used in family studies of both GTS and OCD.

All diagnoses were made by use of the best-estimate approach (Leckman et al. 1982) according to our standard protocol. Before the initial diagnostic estimate was made, separate files for each individual were prepared. These files contained all available information about the individual, including the completed interview packet and medical records, when available. All of this information was reviewed by three clinicians who independently made diagnostic assessments. All three diagnosticians were blind to prior diagnoses and individuals' relationships to the probands. Each interview was evaluated by two raters. The best estimates of the two diagnosticians were then compared. The rate of agreement between any two diagnosticians was very high ($\kappa = 0.97$) for the diagnosis of GTS. When there was disagreement between the two raters who had evaluated the same person, the individual files were reviewed by the third diagnostician, and a final consensus diagnosis was assigned. These consensus diagnoses were then compared with the diagnosis assigned by the clinician at the site where the family was recruited. If there were differences, the clinical materials were reviewed via a conference call, and an attempt was made to reach consensus. If there was still disagreement, more data were requested to help resolve the differences. If there was still disagreement after more data were obtained, the family was removed from the sample.

To make the dichotomous rating of the presence of significant hoarding symptoms, we judged hoarding symptoms to be present when one or both of the hoarding items on the Y-BOCS symptom checklist were rated as present by the experienced clinician. In addition to treating hoarding as a dichotomous outcome, we also considered it as a quantitative trait that was derived from a factor analysis, on the basis of an earlier study of 292 individuals with OCD diagnosed by use of item endorsements from the Y-BOCS symptom checklist (Leckman et al. 1997). The factor loadings and algorithm derived by Leckman et al. (1997) were used to calculate the hoarding-factor scores for the present sample.

DNA Markers

The panel of markers genotyped included 370 DNA markers with an average spacing of 9.1 cM in the male meiotic map on 22 autosomal chromosomes. A detailed description of the markers and map is given by TSAICG (1999).

Data Analysis

Allele frequencies for the genetic markers were established by gene counting in genotyped parents. For each sib pair, the identity-by-descent (IBD) distribution was estimated by single-point and multipoint analyses by use of the MAPMAKER-SIBS and GENEHUNTER programs (Kruglyak and Lander 1995). In the single-point analysis, the IBD distribution was estimated on the basis of the marker genotypes for each marker individually. In the multipoint analysis, maximum-likelihood scores (Risch 1990) were computed for 4,000 different locations relative to the markers (average step size <1 cM). In the estimation of the IBD distribution, only inheritance vectors that were consistent with Mendelian inheritance were considered.

Several analytic approaches have been applied to the sib-pair analyses, as well as the analyses of nuclear families. The main results reported here are from the analysis of the quantitative hoarding-factor score. As mentioned in the "Introduction" section, Leckman et al. (1997) reported four OCD factors, the last of which is the hoarding factor. To examine the unique variability of the hoarding factor, we obtained a residual factor by regressing the original hoarding factor on the other three factor scores. Using the computer program POINTER (Lalouel 1983), J. F. Leckman, D. L. Pauls, H. Zhang, M. C. Rosario-Campos, L. Katsovich, K. K. Kidd, A. J. Pakstis, J. P. Alsobrook, M. M. Robertson, W. M. McMahon, J. T. Walkup, B. J. M. van de Wetering, R. A. King, D. J. Cohen, and the TSAICG (unpublished data) performed a complex segregation analysis for the hoarding-factor score, as a part of an effort to examine the hypothesis that there is transmission of these OCD factors in families and that that transmission is consistent with genetic modes of inheritance. Significant evidence for genetic transmission was obtained for the original hoarding factor, with a possibly recessive inheritance, although the segregation analyses did not suggest evidence for any kind of genetic transmission of the residual hoarding factor.

Linkage analyses of the quantitative hoarding-factor score were completed using the variance-component model for the nuclear families in GENEHUNTER and the traditional and revisited Haseman-Elston sib-pair methods (Haseman and Elston 1972; Elston et al. 2000). In the traditional Haseman-Elston method, the squared

difference between the phenotypes of the two siblings is regressed against their IBD sharing. In the revisited method, the mean corrected cross-product of the sibling traits is used as the dependent variable. Elston et al. (2000) indicate that the newer method is based on a test statistic that has better-understood asymptotic properties and yields better power. Furthermore, as has been noted in the literature, analysis by use of a quantitative phenotype is potentially more powerful than are analyses by use of qualitative phenotypes (Risch and Zhang 1995; Zhang and Risch 1996).

Because the status of hoarding is the chief contributor to the hoarding factor, this phenotype was also examined as a dichotomous trait. Analyses were done with both GENEHUNTER and the RTREE program, which was developed by one of the authors (H.Z.) and is available to the public from his Web site (Zhang Lab of Statistics and Bioinformatics). The RTREE program is based on a recursive-partitioning procedure described by Breiman et al. (1984) and Zhang and Singer (1999). Zhang and Bonney (2000) and Zhang et al. (2001*a*) have explored the potential use of this method in genetic linkage and association studies. Others have also noted the great promise of these techniques in genetic studies (Rao 1998; Shannon et al. 2001). The most attractive features of this method are (1) the ability to accommodate a practically arbitrary number of markers together with environmental factors and (2) the ability to identify potential epistatic (i.e., gene-gene) and gene-environment interactions. Although the method is well established in the statistical literature and the field of machine learning, it is still a novel approach in genetic studies. The key idea is that, in sib-pair analyses, genetic sharing between sib pairs is used to predict the distribution of the numbers of the concordant (both unaffected or both affected are treated as two different concordances) and discordant sib pairs. If a marker is linked to a disease locus, a high-level IBD sharing is expected to result in more concordant sib pairs. Unlike the Haseman-Elston model, the relationship can be simply monotonic, rather than having to be linear (Zhang et al. 2001*a*). In association studies, if some particular alleles are associated with an increased likelihood of a certain condition, the excess level of those allele frequencies should have predictive power to discriminate between the normal condition and the abnormal condition (Zhang and Bonney 2000). In both linkage and association analyses, the recursive-partitioning process stratifies the study sample, on the basis of the genetic information (as well as environmental variables when included in the analyses), into a number of smaller subsamples, in each of which the condition of interest for all observational units is similar (or, ideally, the same). The similarity is usually measured by an entropy function of the distribution of the condition (Breiman et al. 1984; Zhang and Singer 1999; Zhang and Bonney 2000; Zhang et al. 2001*a*). No ascertainment correction was employed for these analyses, since the ascertainment was based on the presence of GTS and not hoarding or OCD.

Results

Results of the analyses of these data suggested linkage to three regions on three chromosomes (4q, 5q, and 17q). Among different analytic methods, the significance levels are largely consistent, regardless of whether the original or the residual hoarding-factor scores were used. Specifically, in the region of 4q34-35, the best significance levels are .003, for the original factor score, and .0007, for the residual score, both of which are based on the nonparametric Z score computed by GENEHUNTER. In the region of 5q35.2-35.3, the best significance levels are .000002, for the original factor score, and .000003, for the residual score, both of which are calculated by the revisited Haseman-Elston method. In the region of 17q25, the best significance levels are .00002, for the original factor score, and .00006, for the residual score, both of which are calculated by the revisited Haseman-Elston method. A graphical presentation of the nonparametric Z scores of these results is provided in figure 1.

As noted above, the status of hoarding as a binary-outcome variable was also examined. Analyses with GENEHUNTER did not reveal any evidence of linkage for this binary trait. The power of GENEHUNTER is markedly reduced by the use of this dichotomized trait. However, by use of the RTREE program, some consistent evidence emerged from both linkage and association analyses on chromosomes 4q and 5q. The tree structure produced from the genomewide scan is shown in figure 2. In the top circle, the so-called "root node" (labeled "1") includes all 77 sib pairs (26 concordant for hoarding, 28 discordant for hoarding, and 23 concordant for being unaffected with hoarding). The IBD sharing from marker D5SMfd154 is first used to partition the sib pairs into two sets. As shown in table 1, this marker is in the region where other methods identified significance evidence of linkage to the quantitative hoarding score. The 62 sib pairs with IBD <1.9 at this marker are assigned to the left circle, the so-called "left daughter node" (labeled "2"). The remaining 15 sib pairs are assigned to the right box, the so-called "right daughter node" (labeled "3"). When IBD can be uniquely defined, the sib pairs in node 3 share exactly the same alleles at this marker. The alleles that are shared by the sib pairs in node 3 are shown in table 2. The first four alleles are shared most among seven unaffected sib pairs, and the last three alleles are mostly shared among eight affected sib pairs. The second split, which applies to the 65 sib

pairs in node 2, uses the IBD sharing at D5S408, which is next to D5SMfd154. This reaffirms the evidence of linkage in the same region. The eight sib pairs in node 4 do not share any allele at this marker, suggesting that it could be either a vulnerability allele or a protective allele. The 54 sib pairs in node 5 share one or both alleles. Finally, node 5 is further divided into nodes 6 and 7 through D4S1652, which is also within the vicinity where linkage to the quantitative-trait locus is suggested. The overall disparities in alleles that are shared among different types of sib pairs for the three selected markers are shown in table 2.

Discussion

Hoarding is likely to be an evolutionarily conserved trait that, in times of adversity, was associated with increased survival and reproductive fitness. However, extreme forms of this trait are associated with marked disability and poor treatment response (Black et al. 1998; Mataix-Cols et al. 1999).

The current analyses provide evidence that alleles shared at specific loci on 4q, 5q, and 17q are associated with this trait. The 4q site is in proximity to D4S1625, which was identified by the TSAICG (1999) as a region linked to the GTS phenotype. The other two regions, 5q and 17q, show the strongest evidence for linkage and have not been previously identified as showing promise in kindreds with GTS or OCD. Future studies will need to evaluate this and other obsessive-compulsive–related quantitative traits in large families segregating for GTS and/or early-onset OCD, by use of highly informative marker sets in these regions of the genome. High-density mapping within these regions and replication studies with the additional GTS-affected sib pairs may also be promising endeavors.

Thus far, only one genome scan of early-onset OCD has been reported. This preliminary report found a possible vulnerability locus on 9p (G. L. Hanna, J. Veenstra-VanderWeele, N. J. Cox, M. Boehnke, J. A. Himle, and G. C. Curtis, personal communication); however, a subsequent study failed to find evidence of linkage disequilibrium at a well-characterized locus in that chromosomal region (Veenstra-VanderWeele et al. 2001). Although virtually all of the siblings with OCD in this sample had an age at onset <10 years (J. F. Leckman, D. L. Pauls, H. Zhang, M. C. Rosario-Campos, L. Katsovich, K. K. Kidd, A. J. Pakstis, J. P. Alsobrook, M. M. Robertson, W. M. McMahon, J. T. Walkup, B. J. M. van de Wetering, R. A. King, D. J. Cohen, and the TSAICG, unpublished data), we found no evidence of linkage to the hoarding trait of OCD in that 9p region.

The one weakness of this study is the small number of sib pairs. As Risch (1990) has noted, very large samples of sib pairs may be necessary to detect linkage when the relative risk is ≤ 2. In the current study, it is not possible to accurately estimate the relative risk, since the population prevalence for hoarding is not known and since no family studies have been reported in which the recurrence risk for hoarding was reported. The best estimate for the relative risk for OCD that is available comes from two family studies of OCD (Pauls et al. 1995; Nestadt et al. 2000*b*). The population prevalence of OCD has been estimated to be ~2% (Karno et al. 1988). The recurrence risk for OCD is ~11%–12% (Pauls et al. 1995; Nestadt et al. 2000*b*). Thus, the best estimate of the relative risk for OCD is ~5.5–6.0. If it is assumed that the proportion of individuals with OCD who have hoarding is constant, then the relative risk for hoarding would also be ~5.5–6.0.

Another limitation is the small number of hoarding items on the Y-BOCS symptom checklist. A dimensional version of Y-BOCS (i.e., DY-BOCS), which should enhance the phenotypic characterization of this trait, is currently under development (Leckman et al. 2000*b*). Alternatively, the Questionnaire for Saving Things, developed by Frost et al. (1995), could be used for this purpose.

The recursive-partitioning methods in genomic scans have lately emerged as flexible and potentially powerful alternatives to the standard approaches (Rao 1998; Zhang and Bonney 2000; Shannon et al. 2001; Zhang et al. 2001*b*). In addition, the success of these methods in the classification of distinct colon-cancer tissues and the methods' potential for the identification of otherwise-obscure epistatic interactions suggest that such techniques may be especially valuable in efforts to identify the risk and protective factors that underlie genetically complex neuropsychiatric disorders (Zhang et al. 2001*b*). Furthermore, the identification of specific allele sharing in specific sib pairs may provide a direct approach to the confirmation of these findings in family-based association studies (Simonic et al. 2001).

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for GTS [MIM *137580] and OCD [MIM 164230])

Zhang Lab of Statistics and Bioinformatics, The, http://peace.med.yale.edu/ (for RTREE)

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Obsessive-Compulsive Symptom Dimensions in Affected Sibling Pairs Diagnosed With Gilles de la Tourette Syndrome

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Obsessive-compulsive disorder (OCD) is an etiologically heterogeneous disorder. Recent factor analyses have consistently identified several symptom dimensions, two of which are associated with increased familial risk for OCD; aggressive, sexual, and religious obsessions and checking compulsions (FACTOR 1) and symmetry and ordering obsessions and compulsions (FACTOR 2). Both of these symptom dimensions are also frequently seen in association with Gilles de la Tourette syndrome (GTS). The purpose of this study was to determine whether these obsessive-compulsive (OC) symptom dimensions are correlated within families (between sibs and between parent-child pairs). Using data collected by the Tourette Syndrome Association International Consortium for Genetics Affected Sibling Pair Study, the authors selected all available GTS sib pairs and their parents for which these OC symptom dimensions (factor scores) could be generated. This group included 128 full sibs and their mothers (54) and fathers (54). Four OC symptom dimension scores were computed for each family member using an algorithm derived from item endorsements from the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) symptom checklist. In addition to a series of univariate analyses, complex segregation analyses were also completed using these quantitative OC symptom dimension scores. FACTOR 1 and FACTOR 2 scores were significantly correlated in sib pairs concordant for GTS. The mother–child correlations, but not father–child correlations, were also significant for these two factors. Segregation analyses were consistent with dominant major gene effects for both FACTOR 1 and FACTOR 2. We conclude that familial factors contribute significantly to OC symptom dimension phenotypes in GTS families. This familial contribution could be genetic or environmental.

KEY WORDS: Gilles de la Tourette syndrome; obsessive-compulsive disorder; quantitative traits; segregation analyses

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INTRODUCTION

Substantial recent advances in molecular genetics have greatly increased the capacity to localize disease genes on the human genome. These methods are now being applied to complex disorders, including Gilles de la Tourette syndrome (GTS) [Tourette Syndrome International Consortium for Genetics, 1999]. One of the major difficulties in the application of these approaches is the likely etiologic heterogeneity of GTS and related phenotypes. Heterogeneity reduces the power of gene-localization methods, such as linkage analysis [Zhang and Risch, 1996; Gu et al., 1998; Alcais and Abel, 1999]. Etiologic heterogeneity may be reflected in phenotypic variability, thus it would be highly desirable to dissect the syndrome, at the level of the phenotype, into valid quantitative heritable components.

Obsessive-compulsive (OC) symptomatology is observed frequently in patients with GTS [Pauls and Leckman, 1986; Pauls et al., 1991; Swerdlow et al., 1999; Robertson, 2000]. Factor analytic studies of OCD patients have identified at least four OC symptom dimensions [Baer, 1994; Leckman et al., 1997, 2001; Mataix-Cols et al., 1999; Summerfeldt et al., 1999; Cavallini et al., 2002]. One dimension (FACTOR 1) is characterized by aggressive, sexual, and religious obsessions and checking compulsions. FACTOR 2 is characterized by symmetry and ordering obsessions and compulsions. FACTOR 3 includes contamination obsessions and cleaning/washing compulsions, and the fourth factor (FACTOR 4) includes hoarding obsessions and compulsions. Preliminary data supporting the validity of these dimensions come from longitudinal studies [Mataix-Cols et al., 2002] as well as functional brain imaging [Rauch et al., 1998; Phillips et al., 2000] and treatment studies [Black et al., 1998; Mataix-Cols et al., 1999]. Preliminary studies also provide evidence for the orderly emergence of similar compulsive traits in normally developing children [Evans et al., 1997; Zohar and Felz, 2001].

Although these studies suggest that these dimensions are stable over time and may have biological validity, there has been little examination of their familial or genetic nature. At the present time, we are aware of only one study that has examined the relationship of these factors to familial risk [Alsobrook et al., 1999]. In that study, the relatives of OCD probands who had high scores on FACTOR 1 or FACTOR 2 were at greater risk for OCD than were relatives of probands who had low scores on those factors. To date there have been no published studies done that examine whether the OC symptom dimensions are themselves familial.

Families of affected sib pairs diagnosed with GTS are ideally suited for efforts to identify the genetic loci responsible for these quantitative OC phenotypes. First, the early-onset OCD observed in these families is likely to be etiologically more homogenous [Pauls et al., 1995; Nestadt et al., 2000]. Second, the two symptom dimensions associated with higher familial risk of OCD (FACTORS 1 and 2) are also frequently observed in patients with GTS [Leckman et al., 1997; Swerdlow et al., 1999; Robertson, 2000]. Third, the use of quantitative traits that are familial provides a more powerful approach than analyses using categorical diagnostic (qualitative) outcomes [Zhang and Risch, 1996; Alcais and Abel, 1999; Elston et al., 2000].

We addressed the question of familial factors underlying the OC symptom dimensions commonly seen in GTS by examining affected sib pairs and their parents ascertained in the Tourette Syndrome Association International Consortium for Genetics Affected Sibling Pair Study [Tourette Syndrome International Consortium for Genetics, 1999]. The purpose of the current study was to test the hypothesis that pairs of GTS relatives resemble one another for each of the four quantitative OC symptom dimensions at greater than chance expectations. To investigate the possible transmission of these quantitative OC symptom dimensions, complex segregation analyses were also carried out.

MATERIALS AND METHODS

Sample

All families consisted of at least two sibs affected with GTS. Families were not included in the analyses reported here if both parents were affected with GTS or if one parent had GTS, chronic motor or vocal tic disorder (CT), OCD, or subclinical OCD and the other parent also received a diagnosis of CT, OCD, or subclinical OCD. All diagnoses were made using DSM-III-R criteria. The criteria for subclinical OCD were the same as those used to make a diagnosis of OCD, except that the individual did not perform the compulsions or obsessions for at least an hour, or did not experience them as ego dystonic, or did not report any impairment. These were the same criteria used in the family study of OCD reported by Pauls et al. [1995]. Written informed consent was obtained for all participants after the procedures had been fully explained. Children were also asked to assent to participate in the study in the presence of their parents after the purpose of the study, the nature of the interviews, and the blood collection procedures were described in age-appropriate language.

Phenotypic Evaluation

When a family entered the study, information concerning both affected sibs and their parents was collected in a two-stage process. The initial stage consisted of the collection of information concerning symptoms associated with GTS and OCD using a self-and-family report based on the tic inventory and ordinal severity scales of the Yale Global Tic Severity Scale [Leckman et al., 1989] and the symptom checklist and ordinal scales of the Yale-Brown Obsessive-Compulsive Scale [Y-BOCS, Goodman et al., 1989]. Earlier versions of these instruments have been used in prior studies of individuals with GTS and OCD and have been shown to have good agreement with expert clinician ratings of tic and OC symptom severity [Leckman et al., 1993, 1994; Swerdlow et al., 1999]. In a second stage, an experienced clinician reviewed these symptom ratings with each family member to insure their accuracy and validity.

These instruments are currently being used in family studies of both GTS and OCD.

Factor scores were computed using an algorithm derived from an earlier study of 292 individuals with OCD and based on item endorsements from the Y-BOCS Symptom Checklist. This factor analysis is described more fully in Leckman et al. [1997]. Briefly, a principal components factor analysis with a Varimax rotation was carried out on symptom counts from the 13 a priori categories in the Y-BOCS symptom checklist in two independent groups of patients with OCD ($N = 208$ and $N = 98$) [Leckman et al., 1993, 1994, 1995; Pauls et al., 1995]. The two data sets yielded nearly identical results. Four factors, obsessions and checking, symmetry and ordering, cleanliness and washing, and hoarding, emerged in each data set, in total accounting for more than 60% of the variance. The algorithm used to generate the factor scores in this study was based on the category-specific coefficients, derived from the total sample of OCD subjects, and reported in Table II of that earlier study [Leckman et al., 1997]. Specifically, the number of items endorsed for each of the 13 symptom categories by each participant in this study was multiplied by the respective category-specific coefficients from the earlier study to generate the four factor scores.

Best-Estimate Diagnoses

All diagnoses were made using the best-estimate approach [Leckman et al., 1982]. The best-estimate procedure used in the present study followed a standard protocol. Before the initial diagnostic estimate was made, separate files for each individual were prepared. These files contained all available information about the individual, including the completed interview packet and medical records, when available. Three clinicians (Bv dW, Rotterdam site; WM, Utah site; and RAK, Yale site) reviewed all available information and independently made diagnostic assessments. All three diagnosticians were blind to the prior diagnosis of the individual and to his/her relationship to the proband. Two raters evaluated each interview. If they judged DSM-IV criteria to be satisfied, then they rated that diagnosis as "definite." If they judged DSM-IV criteria to be satisfied save one criterion, then they rated that diagnosis as "probable." The best estimates of the two diagnosticians were then compared. When there was disagreement between the two raters, the third diagnostician reviewed the individual files and a final consensus diagnosis was assigned. These consensus diagnoses were then compared to the diagnosis assigned by the clinician at the site where the family was recruited. If there were differences, the clinical materials were reviewed via a conference call and consensus was reached if possible. If there was still disagreement, more data were requested to help resolve the differences. If there was still disagreement, the family was removed from the sample.

Statistical Analyses

Because the original factor scores were normally distributed [Leckman et al., 1997], resemblance in sib and relative pairs for quantitative traits was examined by standard parametric analyses of association and analyses of covariance. Because this analysis contained non-independent sib pairs from sibships containing three or more affected individuals, we repeated all analyses, using just the two oldest sibs from sibships with three or more affected members. Age was included as a covariate in the univariate models.

Complex segregation analyses were carried out to examine the hypothesis that there is transmission of OCD within families and if that transmission is consistent with genetic modes of inheritance. Analyses were carried out using the unified model as implemented in the computer program POINTER [Lalouel et al., 1983]. The unified model allows for the possible contributions of both major genetic and polygenic loci (the so-called mixed model). Specific genetic models are hierarchical and were examined by comparing the maximum likelihood estimates using a χ^2 test. In these analyses four parameters were estimated: 1) Q, the frequency of the putative major susceptibility allele; 2) D, the degree of dominance of that allele; 3) H, the heritability of the polygenic component contributing to the expression of OCD; and 4) T, the effect of the major susceptibility allele in the population. In addition, V, stands for the variance of the quantitative trait and, U, the mean value of the quantitative trait given the phenotype. For the factor scores the variance should be 1.0 and the mean should be 0.0. No ascertainment correction was employed for these analyses because the ascertainment was based on the presence of GTS and not the value of these factors.

By taking twice the difference between the maximum log likelihood estimates for each model we compared competing genetic models. This difference in log likelihoods is distributed as a χ^2 statistic with degrees of freedom equal to the difference in the number of estimated parameters in the two models. For example, four parameters are estimated when fitting the Mendelian mixed model and only one is estimated when fitting the polygenic model. Twice the difference between the log likelihoods for the mixed and polygenic model is distributed as a χ^2 with 3 degrees of freedom.

First, we compared the model of "No Transmission" with the mixed model assessed evidence for transmission. If twice the difference in log likelihoods did not yield a significant χ^2 statistic, we concluded that there was no evidence for vertical transmission and no further comparisons were made. If the difference in log likelihood yielded a significant χ^2 statistic, it is taken as evidence for possible vertical transmission and further comparisons were made. The next two comparisons examined: 1) if models of a major gene alone (no polygenic background) were sufficient to account for the presumed vertical transmission; and 2) if models of polygenic inheritance (no major locus) were consistent with the pattern of transmission.

Evidence for a major locus component in transmission was assessed by comparing the likelihood of this model to that of the likelihood of the mixed model. If the difference in twice the log likelihood was not significant, the major locus hypothesis was not rejected. If at the

TABLE I. Gilles de la Tourette Syndrome Sibships Studied for Obsessive-Compulsive Symptoms

Number of siblings with GTS	Number of sibships ($n=54$)	Number of siblings $n=128$	Number of mothers $n=54$	Number of fathers $n=54$
Two	38	76	38	38
Three	12	36	12	12
Four	4	16	4	4

same time, then similar comparison between the polygenic hypothesis and the mixed model hypothesis could be rejected, then a mode of inheritance that includes a major locus with no polygenic background would be the most parsimonious. If both major locus and polygenic hypotheses can be rejected, then a mode of transmission with both a major locus and polygenic background (i.e., the mixed model) would be the most parsimonious.

RESULTS

Sample

The 236 subjects came from 54 families (Table I). Thirty-eight of the families contained two sibs affected with GTS. Twelve families contained three sibs affected with GTS. Four families had four affected sibs. One hundred and sixty-two subjects (68.6%) were judged to have probable or definite GTS. Ninety-seven subjects (41%) were judged to have probable or definite OCD. Table II presents the clinical characteristics of GTS-affected sib pairs and their parents.

Sib and Parental Resemblance for Obsessive Compulsive Symptom Dimensions

We were able to compute four OC symptom dimensions (FACTORS 1–4) for all 236 subjects. The sibs resembled each other significantly with respect to FACTOR 1 and FACTOR 2 scores (Table III). We repeated this analysis after removing non-independent sib pairs. The results were similar, with a significant correspondence between sib pairs with respect to FACTOR 1 and FACTOR 2 (Table III). Partial correlations were also significant such that sib 1's FACTOR 1 score was significantly related to sib 2's FACTOR 1 score even after controlling for the effects of sib 1's and sib 2's FACTOR 2 scores (data not shown). Similarly, the partial correlations for FACTOR 2 were also significant even after controlling for the effects of sib 1's and sib 2's FACTOR 1 scores (data not shown).

The mothers, but not fathers, resembled their affected children with respect to FACTOR 1 and FACTOR 2 scores (Table III). When we repeated this analysis after removing non-independent sib pairs, the results were similar, with a significant mother–child correspondence with respect to just FACTOR 1 and FACTOR 2 (Table III). Partial correlations were also significant such that both sib 1's and sib 2's FACTOR 1 scores were significantly related to the mother's FACTOR 1 score even after controlling for the effects of sib 1's or sib 2's and the mothers' FACTOR 2 scores (data not shown). Similarly, the partial correlations for FACTOR 2 were also significant even after controlling for the effects of either sib's and the mother's FACTOR 1 scores (data not shown).

Because the mother served as the primary informant for the prepubertal subjects, these correlations were computed again using only the data from 17 sibs pairs where both were above the age of 15 years. The sib–sib correlations for FACTOR 1 and FACTOR 2 scores were in same range as for the entire set of sib pairs (0.38 and 0.39, respectively, $P < 0.10$). Interestingly, the FACTOR 4 scores also approached significance (0.42, $P = 0.09$). The mother–child correlations for these older sibs were also comparable to those found in the entire set of

TABLE II. Clinical Characteristics of Gilles de la Tourette Syndrome Affected Sibling Pairs and Their Parents

	Eldest sibling ($N=54$)	2nd Eldest sibling ($N=54$)	Mother ($N=54$)	Father ($N=54$)
Male, *n* (%)	38 (70)	42 (78)	0 (0)	54 (100)
Mean age, years (SD)	14.8 (7.1)	13.6 (7.7)	42.8 (7.5)	44.3 (7.6)
GTS diagnosis: *n* (%)				
Definite	43 (80)	46 (85)	14 (26)	11 (20)
Probable	11 (20)	8 (15)	3 (7)	6 (3)
Age of tic onset, years (SD)	5.7 (2.3)	5.6 (2.6)	7.6 (3.3)	7.6 (2.0)
OCD diagnosis: *n* (%)				
Definite	23 (43)	22 (41)	14 (26)	4 (7)
Probable	8 (15)	7 (13)	4 (7)	3 (6)
OCD age of onset, years (SD)	6.7 (3.5)	6.7 (3.4)	10.3 (3.6)	11.5 (9.6)
SSRI medication history: *n* (%)				
Ever	19 (35)	21 (39)	17 (31)	13 (24)
Current	13 (24)	15 (28)	10 (19)	8 (15)
YGTSS-total tic score				
Current (SD)	16.2 (10.7)[a]	16.2 (10.0)[b]		
Y-BOCS-total score				
Current (SD)	17.6 (7.9)[c]	17.4 (5.9)[d]		

[a] $n = 41$.
[b] $n = 40$.
[c] $n = 22$.
[d] $n = 18$.

TABLE III. Pearson Correlations Between Obsessive-Compulsive Symptom Factor Scores†

OC factor[a]	Sib1–Sib2	Sib1-mother	Sib1-father	Sib2-mother	Sib2-father
Factor 1	0.41*, 0.41*	0.64*, 0.46*	0.13, 0.11	0.36*, 0.24	0.01, 0.15
Factor 2	0.38*, 0.33***	0.41*, 0.25****	0.00, 0.20	0.31**, 0.35**	−0.01, 0.03
Factor 3	0.11, 0.08	0.26***, 0.08	0.04, 0.09	0.26***, 0.25****	−0.11, −0.22
Factor 4	0.16, 0.15	0.12, −0.06	0.05, 0.08	0.18, 012	−0.06, 0.15

†In each cell the first coefficient is for all sib-pairs and all parent-sib pairs. The second coefficient is for the oldest two sibs and their parent-sib correlations.
OC, obsessive-compulsive.
[a]OC Factors scores were generated based on an algorithm developed in a study of 292 patients with OC disorder [Leckman et al., 1997]. Factor 1 includes aggressive, sexual, religious and somatic obsessions and checking compulsions. Factor 2 includes obsessions of symmetry and exactness and ordering, counting, doing and redoing compulsions. Factor 3 includes obsessions of cleanliness and washing compulsions. Factor 4 includes obsessions and compulsions related to hoarding.
*$P < 0.0001$.
**$P < 0.01$.
***$P < 0.05$.
****$P < 0.10$.

mother–child pairs (FACTOR 1 [mother–sib 1: 0.84, $P < 0.0001$; mother–sib 2: 0.53, $P = 0.03$], FACTOR 2 [mother–sib 1: 0.63, $P < 0.007$; mother–sib 2: 0.00, NS] and FACTOR 4 [mother–sib 1: 0.57, $P < 0.02$; mother–sib 2: 0.43, $P = 0.09$]).

In a further effort to examine the relative effect of parent-child vs. sib associations a series of univariate analyses were carried out in which sib 1's factors scores were related to sib 2's factor scores as well as the mother's and father's factor scores while simultaneously controlling for the effects of age. As presented in Table IV, the effects of the mother's FACTOR 1 and FACTOR 2 scores were the most robust predictors of the sib's scores for FACTORS 1 and 2.

Factor Analysis

Using a principal components factor analysis with Varimax rotation, we examined the results when we specified a four-factor model. The four factors that emerged closely resembled the results from the earlier analyses that relied solely on individuals with OCD. This solution also accounted for more than 70% of the variance (Table V).

TABLE IV. Analyses of Variance of Sib1 OC Factor 1 and Factor 2 Scores*

Dependent measure	Independent variables	F (1, 83), P-value[a]	F (1, 43), P-value[b]
S1 Factor 1	Sib1 age	8.71, .004	2.15, NS
	Sib2 age	0.128, NS	0.000, NS
	Mother's age	0.618, NS	0.01, NS
	Father's age	3.60, .06	0.39, NS
	Sib2 Factor 1	5.44, .02	3.31, .08
	Mother Factor 1	35.89, .0001	9.50, .004
	Father Factor 1	0.05, NS	0.00, NS
S1 Factor 2	Sib1 age	1.98, NS	2.59, NS
	Sib2 age	0.303, NS	0.79, NS
	Mother's age	5.90, .017	0.05, NS
	Father's age	6.91, .01	0.14, NS
	Sib2 Factor 2	5.31, .02	2.28, NS
	Mother Factor 2	11.32, .001	2.72, .10
	Father Factor 2	1.08, NS	2.22, NS
S1 Factor 3	Sib1 age	0.002, NS	0.84, NS
	Sib2 age	3.56, .06	0.07, NS
	Mother's age	5.14, .03	0.89, NS
	Father's age	8.78, .004	1.81, NS
	Sib2 Factor 3	0.007, NS	0.37, NS
	Mother Factor 3	2.12, .15	0.26, NS
	Father Factor 3	1.42, NS	1.16, NS
S1 Factor 4	Sib1 age	2.91, .09	4.79, .04
	Sib2 age	0.01, NS	0.80, NS
	Mother's age	0.18, NS	0.03, NS
	Father's age	1.42, NS	0.52, NS
	Sib2 Factor 4	0.13, NS	016, NS
	Mother Factor 4	0.40, NS	0.17, NS
	Father Factor 4	0.33, NS	0.22, NS

*ANCOVAs in which we evaluated the effect of the Sib2's and the parents' Factor scores on Sib1's Factor scores while simultaneously controlling for age.
[a]This analysis was performed using all available S1 pairs ($N = 91$).
[b]This analysis was performed using just the eldest two siblings ($N = 51$).

TABLE V. Varimax Rot ated Factor Structure for Yale-Brown Obsessive Compulsive Scale Symptom Checklist Category Scores

Symptom category	Factor loading[a]			
	Obsessions and checking	Symmetry and ordering	Cleanliness and washing	Hoarding
Aggressive obsessions	**0.62**	0.18	0.49	0.18
Contamination obsessions	0.43	0.27	**0.70**	0.05
Sexual obsessions	**0.83**	0.18	0.18	0.11
Hoarding obsessions	0.09	0.19	0.19	**0.87**
Religious obsessions	**0.66**	0.22	0.21	0.22
Obsessions of symmetry	0.23	**0.76**	0.19	0.23
Somatic obsessions	**0.58**	0.18	0.49	0.15
Cleaning compulsions	0.02	0.08	**0.70**	−0.03
Checking compulsions	0.22	0.45	**0.68**	0.29
Repeating rituals	0.05	0.33	**0.68**	0.30
Counting compulsions	0.41	**0.56**	0.18	0.21
Ordering and arranging	0.12	**0.83**	0.29	0.13
Hoarding and collecting	0.33	0.23	0.06	**0.78**
% of variance explained	21.5	16.7	21.3	13.6 Sum = 72.1%

[a]Robust loadings (greater than 0.50) are printed in bold.

Segregation Analyses

Results of segregation analyses using the four factors as quantitative phenotypes are presented in Table VI. Significant evidence for genetic transmission was obtained for all factors. Of interest is that the specific mode of transmission differed for specific factors. The most parsimonious solution for each factor suggests genes of major effect. For FACTORS 1 and 2 the transmission was consistent with dominant inheritance whereas for FACTORS 3 and 4 the most parsimonious solution was consistent with recessive inheritance.

DISCUSSION

The findings in this study suggest that at least some of the OC symptom dimensions are heritable. First, for FACTORS 1 and 2, there was a significant correlation both between sib pairs and mother–child pairs. To our knowledge, this is the first report of familial resemblance for these quantitative phenotypes in patients with GTS, OCD, and closely related disorders. Second, these correlational findings are strengthened by the results of segregation analyses that suggest that the patterns within families are consistent with genetic

TABLE VI. Segregation Analyses of Yale-Brown Obsessive Compulsive Scale Factor Scores*

	V	U	D	T	Q	H	−2Ln(L)
OC Factor 1[a]							
No transmission	0.70 ± 0.07	−0.53 ± 0.01	0.00[e]	0.00[e]	0.00[e]	0.00[e]	283.563
Mixed model	0.94 ± 0.09	−0.04 ± 0.10	1.00[f]	1.57 ± 0.10	0.29 ± 0.08	0.06 ± 0.06	246.950
Single locus model	0.94 ± 0.09	−0.04 ± 0.19	1.00[f]	1.57 ± 0.11	0.29 ± 0.08	0.00[e]	248.029
Polygenic model	0.67 ± 0.07	−0.50 ± 0.02	0.00[e]	0.00[e]	0.00[e]	0.44 ± 0.08	264.975
OC Factor2[b]							
No transmission	0.88 ± 0.08	−0.36 ± 0.02	0.00[e]	0.00[e]	0.00[e]	0.00[e]	308.599
Mixed model	0.84 ± 0.08	−0.13 ± 0.10	1.00[f]	1.49 ± 0.08	0.39 ± 0.07	0.04 ± 0.05	275.565
Single locus model	0.84 ± 0.08	−0.14 ± 0.09	1.00[f]	1.49 ± 0.08	0.39 ± 0.07	0.00[e]	275.818
Polygenic model	0.90 ± 0.10	−0.17 ± 0.10	0.00[e]	0.00[e]	0.00[e]	0.54 ± 0.10	291.243
OC Factor 3[c]							
No transmission	0.39 ± 0.04	−0.56 ± 0.07	0.00[e]	0.00[e]	0.00[e]	0.00[e]	217.381
Mixed model	0.56 ± 0.09	−0.37 ± 0.03	0.01 ± 0.07	1.51 ± 0.10	0.50 ± 0.07	0.04 ± 0.04	169.891
Single locus model	0.55 ± 0.08	−0.37 ± 0.03	0.03 ± 0.06	1.50 ± 0.10	0.52 ± 0.07	0.00[e]	170.617
Polygenic model	0.41 ± 0.04	−0.52 ± 0.01	0.00[e]	0.00[e]	0.00[e]	0.24 ± 0.05	217.362
OC Factor 4[d]							
No transmission	1.09 ± 0.10	0.37 ± 0.07	0.00[e]	0.00[e]	0.00[e]	0.00[e]	333.097
Mixed model	0.97 ± 0.18	1.03 ± 0.22	0.00[f]	1.91 ± 0.07	0.85 ± 0.06	0.01 ± 0.01	287.290
Single locus model	0.97 ± 0.19	1.03 ± 0.23	0.00[f]	1.91 ± 0.07	0.85 ± 0.07	0.00[e]	287.294
Polygenic model	1.09 ± 0.10	0.38 ± 0.08	0.00[e]	0.00[e]	0.00[e]	0.08 ± 0.08	332.643

*V, variance of the quantitative trait; U, mean value of the quantitative trait; D, the degree of dominance of that allele; T, the effect of the major susceptibility allele in the population; Q, the frequency of the putative major susceptibility allele; H, the heritability of the polygenic component; NS, not significant.
[a]No transmission vs. mixed, $\chi^2_{(4)} = 36.613$ ($P < 0.000001$); Mixed vs. single locus, $\chi^2_{(1)} = 1.079$, NS; Mixed vs. polygenic, $\chi^2_{(3)} = 18.025$ ($P < 0.0005$).
[b]No transmission vs. mixed, $\chi^2_{(4)} = 33.034$ ($P < 0.000001$); Mixed vs. single locus, $\chi^2_{(1)} = 0.253$, NS; Mixed vs. polygenic, $\chi^2_{(3)} = 15.678$ ($P < 0.002$).
[c]No transmission vs. mixed, $\chi^2_{(4)} = 47.490$ ($P < 0.000001$); Mixed vs. single locus, $\chi^2_{(1)} = 0.726$, NS; Mixed vs. polygenic, $\chi^2_{(3)} = 47.471$ ($P < 0.000001$).
[d]No transmission vs. mixed, $\chi^2_{(4)} = 45.807$ ($P < 0.000001$); Mixed vs. single locus, $\chi^2_{(1)} = 0.004$, NS; Mixed vs. polygenic, $\chi^2_{(3)} = 45.353$ ($P < 0.0001$).
[e]Parameter fixed for the analysis.
[f]Parameter converged at the boundary, no standard error estimated.

transmission. The most parsimonious results were consistent with the hypothesis that there are genes of major effect underlying each of these OC symptom dimensions. It is important to note that although FACTORS 3 and 4 that did not show a significant correlation between sibs or parents, their transmission patterns within families were consistent with recessive inheritance. For recessive inheritance, the expected correlation between sibs would be 0.25 and between parents and children it would approach zero. Consequently, it is not surprising that the correlations observed for those factors were small and, given the size of our current sample, not statistically significant.

Limits

Although much of the available data are promising, there are many questions yet unanswered concerning the value of a dimensional approach in OCD and related disorders. Principal among these is how best to measure these dimensional traits in patients and populations. Among patient populations the factor structure for these OC symptom dimensions has been remarkably consistent across at least six large studies [Leckman et al., 1997, 2001; Mataix-Cols et al., 1999; Summerfeldt et al., 1999; Cavallini et al., 2002]. The results of the present study offer additional support for the validity of these symptom dimensions. These symptom dimensions appear to be relatively stable over 2-year intervals in adults [Mataix-Cols et al., 2002], but similar studies have yet to be carried out in younger OCD patient groups. Studies of normal populations have yet to be completed although data from normally developing children are consistent with the orderly emergence of these traits [Evans et al., 1997; Zohar and Felz, 2001].

It is also possible that the observed associations between the sib pairs and the mother-child dyads is due in part to a measurement bias in that the mothers typically served as the primary informant in the study. We believe, however, that this may not be a major factor. First, these associations were generally stronger when the analyses were confined to the older sib pairs. Second, the observed associations were generally not present for FACTOR 3, contamination obsessions and cleaning compulsions, traits that would likely be subject to the same biases. Third, the requirement that an experienced clinician validate each symptom also insured the highest standard of accuracy. Finally, the possibility that these findings are the result of non-genetic transmission cannot be excluded given the design of this study.

Other issues include the accuracy of the four-factor solution with some investigators suggesting that FACTOR 1 is divisible within two separate domains: aggressive obsessions and related checking compulsions vs. obsessions with either sexual or religious content [Mataix-Cols et al., 1999].

Future studies using a larger collection of sib pairs will also need to examine gender-specific associations (mother–son, mother–daughter, father–son, father–daughter) in an effort to replicate these findings as well as evaluate possible parent-of-origin effects (genetic imprinting) vs. gender-specific effects. It may also be important to use analytic strategies that would permit the use of data from all sibs and that would also take into account the correlation of observations within families [Hudson et al., 2001]. The selection of the oldest two sibs in this analysis was not wholly an arbitrary decision, however, because their inclusion should provide the best lifetime data regarding the development of OC symptoms.

The results of the segregation analyses should be viewed with caution. For example, because FACTORS 1 and 2 are known to be associated with tic disorders, the ascertainment of the sib pairs was not completely unbiased with regard to two of these quantitative phenotypes. In addition, the estimates of allele frequencies are uniformly high (0.29–0.85) and may reflect some aspect of the unique ascertainment of this sample. In addition, given that the factor scores for FACTORS 1, 2, and 3 were inter-correlated, future analyses should endeavor to estimate the unique variance for each factor as well as the common variance for all four. These residual variances and the common variance could then be used as quantitative phenotypes in future segregation and linkage analyses using larger data sets.

CONCLUSIONS

Common disorders with genetic susceptibilities are likely to involve the action of multiple genes interacting with each other and with environmental factors, making it difficult to localize the specific genetic loci responsible. An important route to the disentangling of this complex inheritance may be through the study of normal variation in quantitative psychological traits. Studies of the symptoms of OCD have led to the identification of at least four such traits. A preliminary study of OCD probands has found evidence that at least two of these factors may be useful in family aggregation studies. This study confirms the promise of these factors, particularly in patients at high risk for GTS. Genome scans are currently underway to utilize these quantitative OC phenotypes in multipoint linkage analyses [Zhang et al., 2002]. Further, if OCD proves to be a multidimensional and etiologically heterogeneous condition, it may be used to develop "state" as well as "trait" assessment instruments based on these symptom dimensions [Leckman et al., 2000]. Such scales could then be used in clinical trials to quantify more precisely the patterns of treatment response. The factors associated with an unfavorable treatment response remain largely unknown. Although global ratings of pretreatment symptom severity appear not to be a useful predictor of response [Steketee, 1993; Ackerman et al., 1994], the differential patterns of response for OCD patients with hoarding symptoms emphasize the potential value of separate severity scales for separate symptom dimensions.

With regard to GTS, these results are consistent with the high rate of OC symptoms reported in GTS families [Pauls and Leckman, 1986; Pauls et al., 1991; Leckman et al., 1997; Swerdlow et al., 1999; Robertson,

2000] and may aid in the identification of autosomal genes that predispose individuals to both GTS and specific forms of OCD [Pauls and Leckman, 1986; Robertson and Gourdie, 1990; Eapen et al., 1993; Gardos et al., 2001].

These dimensions may also be of heuristic value in the context of evolutionary perspectives on psychopathology and the emergence of related behaviors across the OCD spectrum including eating disorders, body dysmorphic disorder, autism, and Prader-Willi syndrome as well as during the course of normal development [Evans et al., 1997; Leckman and Mayes, 1998; Bienvenu et al., 2000; Zohar and Felz, 2001; Halmi et al., in press].

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Evaluation of the Genes for the Adrenergic Receptors α2A and α1C and Gilles de la Tourette Syndrome

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Gilles de la Tourette Syndrome (GTS) has long been known to be familial, and evidence from twin studies indicates that it has a substantial genetic component. Our genome scan of sibling pair families with GTS found evidence suggestive of linkage to several chromosomal locations. On the basis of these findings, we have begun to study additional markers in these regions, with some of the markers located in candidate genes. Two candidate genes stand out in these regions: the adrenergic receptor α1C(1A) (*ADRA1C*) located on chromosome 8p and the adrenergic receptor α2A (*ADRA2A*) located on chromosome 10q. The adrenergic system has been suggested to play a role in GTS based on the reduction of symptoms with the adrenergic receptor agonists, clonidine and guanfacine. We examined the inheritance of polymorphisms in the *ADRA2A* and *ADRA1C* genes in 113 nuclear families identified through a GTS proband. We found no significant evidence for linkage using the transmission disequilibrium test for these two genes. Based on our families, we conclude that these genes are not major genetic factors contributing to the susceptibility to GTS.

KEY WORDS: Gilles de la Tourette syndrome; genetics; adrenergic receptor α1C (α1a); adrenergic receptor α2A; linkage

INTRODUCTION

Gilles de la Tourette syndrome (GTS) is a familial disorder characterized by the presence of both motor and phonic tics. Other disorders are commonly observed in affected individuals and in family members of GTS probands and segregation studies have supported that some of these phenotypes, in particular chronic multiple tics (CMT) and obsessive compulsive disorder, may share a common genetic susceptibility in GTS families [Pauls et al., 1986, 1990, 1991; Walkup et al., 1996]. Symptoms of attention-deficit hyperactivity disorder (ADHD) are common in individuals with GTS and their family members and have been suggested to be an alternative phenotype of GTS susceptibility genes. The genetic link between GTS and ADHD, however, has not been clarified based on segregation studies [Barr and Sandor, 1998].

The search for genetic factors contributing to the susceptibility of GTS has been investigated by a number of groups using linkage and association studies [Barr and Sandor, 1998]. These studies include genome scans for GTS, using multi-generational families [Pakstis et al., 1991; Barr et al., 1999], nuclear families with affected sibling pairs [The Tourette Syndrome Association International Consortium for Genetics, 1999], and studies using pools of DNA from individuals with GTS from the Afrikaner population compared with those of control subjects [Simonic et al., 1998].

Our recent genome scan of 119 affected sibling pairs, carried out as part of an international consortium

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identified several regions that provided some evidence for linkage although the findings did not reach genome-wide significance levels [The Tourette Syndrome Association International Consortium for Genetics, 1999]. One region identified was located on the short arm of chromosome 8 in the region of D8S1106. Additional markers in the region (D8S1130, D8S1145, and D8S136) also provided support for linkage. Another of the regions with a LOD score greater than 1 was located on chromosome 10q in the region of marker D10S1213. Within these two regions, two genes stand out as promising candidates—the adrenergic receptor α1C (*ADRA1C*) and the adrenergic receptor α2A (*ADRA2A*).

The adrenergic system has always been a particularly attractive candidate for the genetic susceptibility to GTS, considering the efficacy of treatment of tics with clonidine and guanfacine [Leckman et al., 1991; Chappell et al., 1995; Lichter and Jackson, 1996]. Clonidine and guanfacine are α-adrenergic agonists that preferentially stimulate the α2 adrenergic receptors. Stimulation of the presynaptic α2 receptors decreases the amount of norepinephrine released into the synapse, suggesting that dysregulation of the adrenergic system may be causally involved in GTS.

The α-adrenergic receptors function in a many physiological processes, including control of blood pressure and flow, digestion, reproduction, pupil diameter, endocrine and metabolic processes, and most importantly for this study, neural modulation and behavior [Docherty, 1998]. The activation of α1-adrenergic receptor promotes vigilance (sustained attention), and selective attention and influences working memory and behavioral activation [reviewed in Sirvio and MacDonald, 1999]. Stimulation of the α2 receptors has been shown to improve cognitive function and distractability and this receptor has been suggested to be involved ADHD [Arnsten et al., 1996].

The *ADRA2A* gene has previously been tested as a susceptibility factor in ADHD using a quantitative approach and ADHD symptom scores in a sample of 274 individuals diagnosed with GTS and 62 controls [Comings et al., 1999]. Of the individuals with GTS, 144 also met DSM-IV criteria for ADHD. That study reported a correlation between scores for ADHD ($P = 0.043$) using regression analysis. However, it did not specifically analyze the *ADRA2A* gene as a susceptibility factor for GTS, so it is not possible to determine whether this finding relates to the susceptibility to GTS or only to the susceptibility to the symptoms of ADHD in the sample. Studies of the *ADRA2A* and *ADRA1C* genes in an ADHD sample without GTS or chronic tics did not find any evidence for linkage of these two genes to the ADHD phenotype [Barr et al., 2001; Xu et al., 2001].

Because ADRA1C and ADRA2A are located in regions with some support for linkage, and because the pharmacological and physiological evidence shows adrenergic receptors to be involved in attentional processes and reduction in tic symptoms in some individuals, we explored the possibility that these genes contribute to the genetic susceptibility to GTS. We genotyped a total of 113 probands with 89 affected siblings for polymorphisms at the ADRA1C and ADRA2A loci and tested for linkage using the transmission disequilibrium test [Spielman et al., 1993].

MATERIALS AND METHODS

Subjects

We genotyped in total 113 small nuclear families (probands, parents and affected siblings) collected through a GTS proband. Seventy-two of the families had more than one affected child with a total of 202 affected children in the analysis (Table I). The majority of these families were collected as part of a multi-site consortium study that was used for the genome scan of affected sib pairs [The Tourette Syndrome Association International Consortium for Genetics, 1999]. Additional families were collected from Canada and Turkey for this study.

Diagnostic Assessment

Canada and international consortium sample. The diagnostic assessment of subjects for this study (probands, siblings, and parents of the affected sibling pair families) has been previously described [The Tourette Syndrome Association International Consortium for Genetics, 1999]. Briefly, information about symptoms associated with GTS and obsessive compulsive disorder was collected using a self- and family-report based on the tic inventory and ordinal severity scales of the Yale Global Tic Severity Scale [Leckman et al., 1989] and the symptom checklist and ordinal scales of the Yale-Brown Obsessive-compulsive scale [Goodman et al., 1989]. Other psychopathology was assessed using the K-SADS [Chambers et al., 1985] for children under 18 and the SCID [Spitzer et al., 1992] for adults. The information was checked by an experienced neuropsychiatrist and complemented by the direct examination of symptoms using the Yale Global Tic Severity Scale and the Yale-Brown Obsessive-Compulsive Scale.

Turkish sample. Children were assessed at the Marmara University Medical School, Development and Neuropsychiatry Unit, Istanbul. A definite diagnosis of GTS and other comorbidities was established by a child psychiatrist according to DSM-IV criteria, using a semistructured interview with child and parents based on the K-SADS. The diagnosis was confirmed by another child psychiatrist blind to the original diagnosis. Turkish versions of the Yale-Global Tic Severity Scale and the Yale-Brown Obsessive Compulsive Scale (child or adult, when appropriate) were also administered for severity ratings.

TABLE I. Summary of Families

	Canadian	Turkish	Consortium
Total families	42	29	42
One affected child	15	26	0
Two affected children	20	3	34
Three affected children	6	0	7
Four affected children	1	0	1
Total children with GTS	77	32	93

Isolation of DNA and Marker Typing

DNA was extracted from blood lymphocytes using a standard high salt extraction method [Miller et al., 1988]. To genotype the Cys492Arg polymorphism of ADRA1C, we amplified a 502 bp fragment using a reaction mixture of 100 ng of genomic DNA, the primers ADRA1C-Pst1 (5′ atg ctc cag cca aga gtt ca 3′) and ADRA1C-Pst2 (5′ tcc aag aag agc tgg cct tc 3′), 1.5 mM $MgCl_2$, and 1 U of Taq polymerase [Shibata et al., 1996]. The polymerase chain reaction (PCR) reaction consisted of an initial denaturation stage of 4 min at 94°C followed by 35 cycles of denaturing at 94°C for 30 sec, annealing at 58°C for 30 sec, and an extension of 72°C for 30 sec. A final extension step of 72°C was added for 10 min after the last cycle. To detect the polymorphism, 10 μl of the PCR product was digested with 8 U of *Pst*I restriction enzyme (New England Biolabs, Beverly MA) for approximately 2 h at 37°C. The alleles were separated on a 2% agarose gel. Allele 1 (C, Cys492) was not cut with the restriction enzyme and was seen as a 501 bp band. Allele 2 (T, Arg492) was cut into two bands of 477 and 25 bp.

To genotype the −1291 promoter region polymorphism for ADRA2A, we amplified a 522 bp fragment by PCR with the primers ADRA2A-*Msp*I F (5′ tca cac cgg agg tta ctt ccc tcg) located at position −1417 and ADRA2A-*Msp*I R (5′ tcc gac gac agc gcg agt t) located at position −913 [Lario et al., 1997]. The modified PCR conditions consisted of 35 cycles of denaturation at 94°C for 40 sec, annealing at 58°C for 40 sec and extension at 72° C for 1 min. PCR products (8 μl) was digested with 5 U of *Msp*I (New England Biolabs, Mississauga, ON, Canada) at 37°C for 2 h. The fragments were separated on a 4.8% polyacrylamide gel followed by silver staining to detect the alleles. The allele designated 1, is detected by a polymorphic band of 174 bp and several constant bands of 165, 116, 62, and 5 bp. The change of C to G reduces the 174 bp fragment into two bands of 121 and 53 bp (designated allele 2).

Statistical Analysis

To test these two genes for linkage, we examined the inheritance of a common polymorphism at each locus using the transmission disequilibrium test (TDT) [Spielman et al., 1993] calculated with the extended transmission disequilibrium test (ETDT) program [Sham and Curtis, 1995]. This test considers parents who are heterozygous for an allele and evaluates the frequency with which an allele is transmitted to an affected offspring compared to the alternative alleles. Under the null hypothesis each allele has an equal chance of being transmitted and deviation from equal transmissions indicates evidence for linkage. We chose the TDT as the statistical test for this study because it allows the use of genotypes from affected siblings. Since we were using the genotypes from the siblings in the analysis the test is more accurately described as a test for linkage as opposed to association. We therefore use the term linkage throughout this paper to describe our results.

For the analysis, the families from Canada and Turkey were analyzed separately to determine if there were site-specific differences. The families from the multi-site consortium were not analyzed individually by collection site because there were not sufficient families collected from any one site to be informative.

RESULTS

We have chosen for this analysis, two single nucleotide polymorphisms (SNPs). Although microsatellite markers are potentially more informative for linkage studies, polymorphisms located within the gene are more likely to have remained in linkage disequilibrium with DNA sequence changes contributing to the phenotype, and therefore may be more useful for studies based on linkage disequilibrium. Further, DNA changes that alter the function or the expression of the protein may potentially be a change contributing to GTS and therefore are of primary interest for testing in our study. For the *ADRA1C* gene, we used a *Pst*I polymorphism identified first by traditional restriction fragment length polymorphism methods [Hoehe et al., 1995] and later as a C to T change located in codon 492 resulting in a change of cysteine to arginine [Shibata et al., 1996]. This amino acid variation is unlikely to cause a change in phenotype, since no differences in receptor function were observed between the two protein variants [Shibata et al., 1996]. The pharmacological characteristics and the receptor-mediated $[Ca^{2+}]_i$ response were found to be desensitized in a similar manner for either of these receptor variants. For the *ADRA2A* gene, we genotyped the C to G polymorphism located at position −1291 upstream from the putative origin of transcription of the *ADRA2A* gene [Lario et al., 1997].

We examined the frequencies of the alleles in the chromosomes of the parents for each sample. The allele frequencies for the *Msp*I polymorphism in ADRA2A are shown in Table II, those for the *Pst*I polymorphism of *ADRA1C* in Table III.

We calculated the TDT for the samples collected in Canada, Turkey, and the consortium sample individually and then combined the results. For the ADRA2A polymorphism, we found no significant evidence for biased transmission of either allele in any of the samples; however, there was a trend for the biased transmission of the 1 allele in the multi-site consortium sample (Table IV). For the ADRA1C polymorphism, the results for the sample collected in Canada showed a trend for biased transmission (see Table V). The number of informative transmissions totaled 76. The Cys492 allele was transmitted 44 times to the probands and affected siblings vs. 32 times that it was not transmitted. For the sample collected in Turkey, there was also a

TABLE II. Allele Frequencies of the ADRA2A-*Msp*I Polymorphism

	Canadian	Turkish	Consortium
Allele 1	0.709	0.798	0.722
Allele 2	0.291	0.202	0.278

TABLE III. Allele Frequencies of the ADRA1C-*Pst*I Polymorphism

	Canadian	Turkish	Consortium
Allele 1	0.466	0.617	0.580
Allele 2	0.534	0.383	0.420

trend for the transmission of the Cys492 allele. Of 27 informative transmissions, the Cys492 allele was transmitted 18 times compared to 9 times that it was not transmitted. No evidence for biased transmission was found in the international consortium sample (Cys492 allele transmitted 33 times vs. 32 times that it was not transmitted). For the combined sample, the number of informative transmissions totaled 162 with the Cys492 transmitted 91 times vs. 71 times that it was not transmitted ($\chi^2 = 2.469$, 1 d.f., $P = 0.116$).

DISCUSSION

For both the *ADRA2A* and *ADRA1C* genes, we did not find significant evidence for linkage in this sample. However, we did note a trend for the Cys492 allele of the *ADRA1C* gene to be transmitted to the affected offspring in the Canadian and Turkish samples. This trend may indicate that a portion of the sample is linked to this gene but our sample lacked sufficient power to identify linkage. The results from our genome scans suggest that there may be substantial locus heterogeneity in GTS [Barr et al., 1999; The Tourette Syndrome Association International Consortium for Genetics, 1999]. If only a few of the families in our sample are linked to these genes, or if the relative risk of the gene to the development of the phenotype is small, a larger sample would be required to identify linkage for any one gene contributing to GTS. However given that the TSA consortium sample used in this study did provide evidence for linkage to this region using an affected sibling pair analysis that is less powerful than methods that rely on linkage disequilibrium, it would be expected that if the marker is in linkage disequilibrium with the susceptibility allele then the TDT analysis would have sufficient power to detect these genes. While it is not possible to precisely determine the power of a sample to detect a particular susceptibility gene because the contribution of each gene to the risk for the development of the phenotype and the mode of inheritance of each gene is unknown, we can estimate that this particular sample of families could detect a susceptibility gene using TDT analysis with a genotypic relative risk of 15 given an autosomal dominant mode of inheritance and disease allele frequency of 0.003 [Chen and Deng, 2001].

It is possible that the *ADRA1C* gene is linked to GTS but the polymorphism that we tested is not in strong linkage disequilibrium with the DNA variant responsible for the phenotype. We screened the coding region of the *ADRA1C* gene in our GTS probands by direct sequencing and single stranded conformational polymorphisms analysis but we found no additional common polymorphism that could be used for linkage analysis. We have not at this time screened the promoter region or the single intron of this gene.

In light of our genome scan on part of this sample, then the criteria needed to accept linkage must be stringent and this trend would fall far short of a genome wide significance level. Therefore, the most likely explanation is that this trend for biased transmission is a chance finding. It is still possible that the 8p and 10q regions are linked to GTS but that these two adrenergic receptors are not the susceptibility genes. The suspected role of the adrenergic system in GTS made these genes logical choices as candidates in these two regions. Further, the previous linkage report of the *ADRA2A* gene and ADHD symptoms in a sample ascertained through a GTS proband supported this gene as a strong candidate in the 10q region. The study of these two genes was therefore our first step in the search for susceptibility genes in these regions and we will continue to study genes in these two regions.

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TABLE IV. TDT Results of the ADRA2A-*Msp*I Polymorphism

	Canadian		Turkish		Consortium		Combined	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
Transmitted	26	22	8	12	39	28	73	62
Not transmitted	22	26	12	8	28	39	62	73
Chi-squares	0.333	0.333	0.800	0.800	1.806	1.806	0.896	0.896
P values	0.564	0.564	0.371	0.371	0.179	0.179	0.344	0.344

TABLE V. TDT Results of the ADRA1C-*Pst*I Polymorphism

	Canadian		Turkish		Consortium		Combined	
	Cys492	Arg492	Cys492	Arg492	Cys492	Arg492	Cys492	Arg492
Transmitted	44	32	18	9	33	32	91	71
Not transmitted	32	44	9	18	32	33	71	91
Chi-squares	1.895	1.895	3.000	3.000	0.015	0.015	2.469	2.469
P values	0.169	0.169	0.083	0.083	0.901	0.901	0.116	0.116

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CNTNAP2 is disrupted in a family with Gilles de la Tourette syndrome and obsessive compulsive disorder

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Abstract

Gilles de la Tourette syndrome (GTS) is a sporadic or inherited complex neuropsychiatric disorder characterized by involuntary motor and vocal tics. There is comorbidity with disorders like obsessive compulsive disorder and attention deficit hyperactivity disorder. Until now linkage analysis has pointed to a number of chromosomal locations, but has failed to identify a clear candidate gene(s). We have investigated a GTS family with a complex chromosomal insertion/translocation involving chromosomes 2 and 7. The affected father [46,XY,inv(2)(p23q22),ins(7;2)(q35–q36;p21p23)] and two affected children [46,XX,der(7)ins(7;2)(q35–q36;p21p23) and 46,XY,der(7)ins(7;2)(q35–q36;p213p23)] share a chromosome 2p21–p23 insertion on chromosome 7q35–q36, thereby interrupting the contactin-associated protein 2 gene (*CNTNAP2*). This gene encodes a membrane protein located in a specific compartment at the nodes of Ranvier of axons. We hypothesize that disruption or decreased expression of *CNTNAP2* could lead to a disturbed distribution of the K^+ channels in the nervous system, thereby influencing conduction and/or repolarization of action potentials, causing unwanted actions or movements in GTS.

Keywords: Gilles de la Tourette syndrome; Contactin-associated protein; Potassium channel; Node of Ranvier; Translocation breakpoint; Chromosome 7; Chromosome 2

Gilles de la Tourette syndrome (GTS) (OMIM 137580) is an inherited neuropsychiatric disorder characterized by repeated simple or complex involuntary motor and vocal tics that begin in childhood and follow a waxing and waning course [1–4].

GTS is also often accompanied by features associated with obsessive compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD), poor impulse control, and other behavioral problems [1,2]. However, the relationships between both ADHD and GTS and OCD and GTS are complex and not yet clear [5]. Evidence is accumulating that an association exists between OCD and GTS [6,7]. Specific symptoms of obsessive–compulsive disorder are found to be similar in GTS with and without OCD and differ from tic-free OCD [8]. These studies suggest that GTS with OCD constitutes a form of GTS, and not of OCD, and reinforce the ideas that at least some forms of OCD are etiologically related to GTS and that GTS + OCD represents a variant phenotype or maybe a more severe form of GTS. At the same time OCD alone in individuals of GTS families might be considered an alternative phenotype or could be due to reduced penetrance of the autosomal dominant mode of inheritance of the disease [9].

Although the cause of GTS is unknown, there is evidence that genetic factors are involved [10,11]. An obvious first approach to search for responsible genes is linkage studies, but due to the complexity of the disorder, until now, results have remained elusive [12]. Several genomic regions have been identified using linkage and association studies point-

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ing to susceptibility loci for GTS, although no clear candidate genes have yet emerged [13–16]. Other regions have been excluded [17,18]. Another approach is the identification and cloning of breakpoints in GTS patients with chromosomal abnormalities. The advantage of this method is that when the interrupted region harbors a specific gene, this may give a direct entry into a biological pathway involved in the etiology of GTS. There is also always the possibility that the breakpoint is coincidental and has no relation to the GTS phenotype. A number of candidate genes have been proposed [19,20], but none of them has yet led to the long-awaited breakthrough. The only gene reported that was interrupted by a breakpoint is IMMP2L [20], the human homologue of the yeast mitochondrial inner membrane peptidase subunit 2; however, its role in the etiology of GTS has not been demonstrated. Candidate regions that have been indicated by chromosomal aberrations include chromosomes 9p [21], 7q22, 18q22 [22], 8q22 [19], and 7q22–q31 [23].

In this paper we describe a family with a complex translocation/insertion/inversion involving chromosomes 2 and 7. The father as well as his two children has GTS characteristics. With karyotyping and fluorescence in situ hybridization (FISH) analysis this complex chromosomal rearrangement has been resolved. All breakpoints have been determined with bacterial artificial chromosome (BAC) clones identifying these sites. The insertion of chromosome 2p22–p23 in chromosome 7q35–q36 interrupts a recently described gene, contactin-associated protein 2 [24], in all three patients.

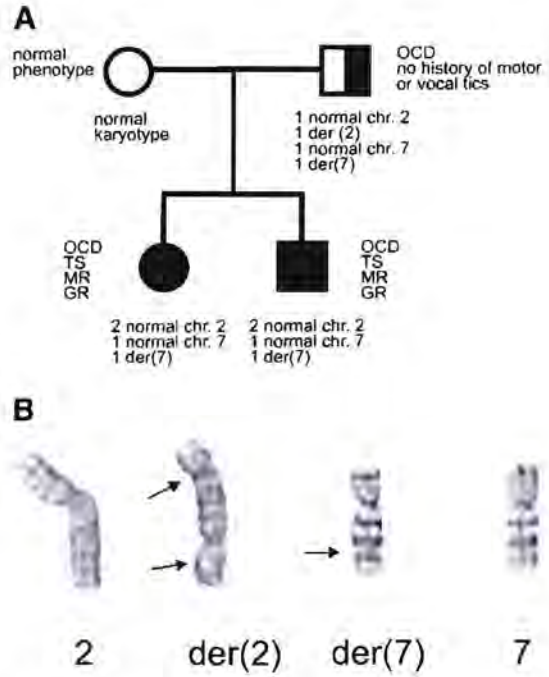


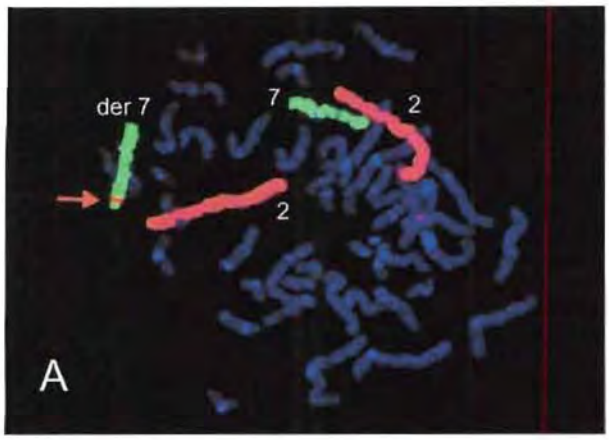
Fig. 1. Family with chromosome 2/7 rearrangement. (A) Pedigree with description of phenotype. (B) Normal and derivative chromosomes 2 and 7 as seen in the karyotype of the father. The children inherited the der(7) from the father. Arrows indicate abnormalities. OCD, obsessive compulsive disorder; TS, Tourette syndrome; MS, mental retardation; GR, growth retardation.

Results

Clinical and cytogenetic evaluation of the family

In Fig. 1A the pedigree of the family is shown. This family was referred to one of us (C.M.) via the Tourette Syndrome Association. A full case report is presented under Materials and methods. In short, the mother is unaffected, the father has OCD. The daughter has Tourette syndrome, OCD, mental retardation, and speech abnormalities. The son has Tourette syndrome, OCD, mental retardation, and some speech abnormalities, but less severe than his sister. In addition both children have growth retardation. The karyotype of the mother was normal, the karyotype of the father showed abnormalities involving one chromosome 2 and one chromosome 7, the children showed two normal chromosomes 2 and one abnormal chromosome 7. The cytogenetic reports derived from the karyotype are for the father 46,XY, inv(2)(p23q22),ins(7;2)(q35–q36;p21p23) and for the daughter 46,XX,der(7)ins(7;2)(q35–q36;p21p23) and son 46,XY,der(7)ins(7;2)(q35–q36;p213p23). Fig. 1B shows the normal and derivative chromosomes 2 and 7 as seen in the karyotype of the father. The children both inherited the normal chromosome 2 and the derivative chromosome 7 from the father.

Fig. 2. Fluorescence in situ hybridization. (A) Chromosomal paint with chromosome 2 in red and chromosome 7 in green. The metaphase shown is of the son's chromosomes. For the father and daughter the same results were obtained, indicating an insertion of part of chromosome 2 into chromosome 7 for all three Tourette patients in the family. (B) Comparative genomic hybridization with DNA from both children. A gain of chromosome band 2p21–p23 is seen. (C) BAC 641E23 (green) crosses the breakpoint of chromosome 7 where the chromosome 2 region (red) is inserted into chromosome 7q35–q36. Chromosome 2 is painted in red. The metaphase shown is of the son's chromosomes. (D) BAC 490M8 (red) indicates the proximal breakpoint of the chromosome 2 deletion/insertion. Signal is present on der2q22 and der7q35 (red arrows) and on the normal chromosome 2p23. In addition a homologous signal is present on chromosome 7q11–q21 (white arrows). The metaphase shown is of the father's chromosomes. (E) The upper breakpoint of the inversion, on the short arm of chromosome 2, is represented by BAC clone 342D1, localized on chromosome 2p23 of the normal chromosome 2 and on chromosome 2p23 and 2q22 of the abnormal chromosome 2. The metaphase shown is of the father's chromosomes. (F) The lower breakpoint of the inversion, on the long arm of chromosome 2, is represented by BAC clone 432O12. This clone is localized on chromosome 2q22 of the normal chromosome 2 and on chromosomes 2q22 and 2p23 of the abnormal chromosome 2. The metaphase shown is of the father's chromosomes. (G) FISH to show the order of BAC clones on chromosome 2p using BAC 560C7 (in green) and BAC 742M10 (in red). The metaphase shown is of the father's chromosomes. On der(7) BAC 742M10 is inserted, on der(2) BAC 560C7 is inverted.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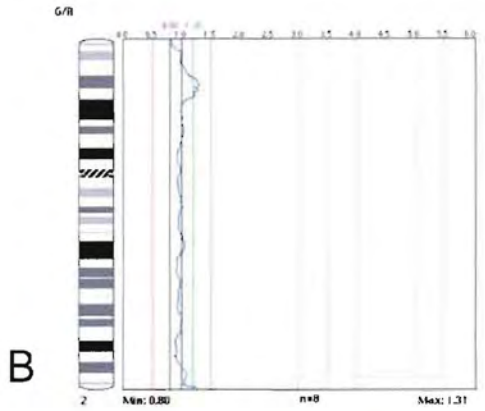
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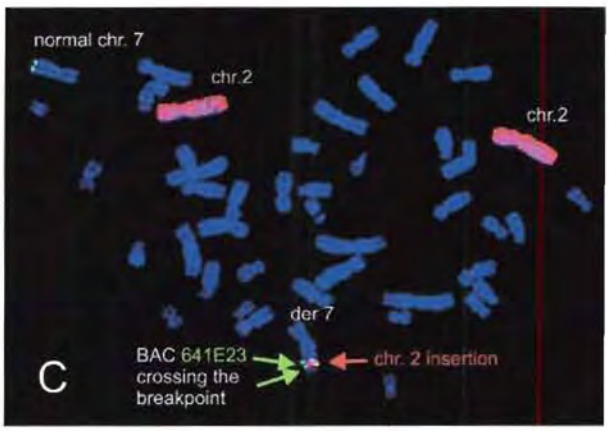
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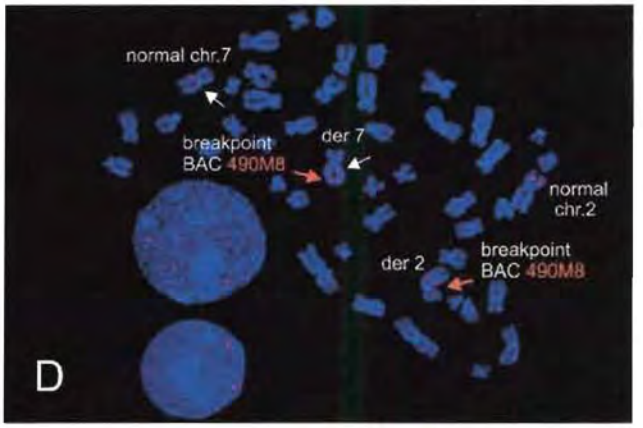
C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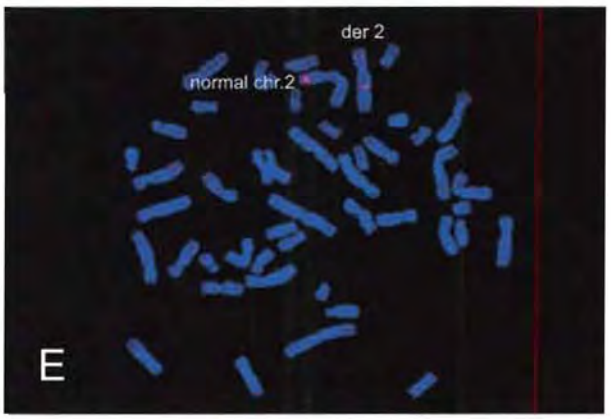
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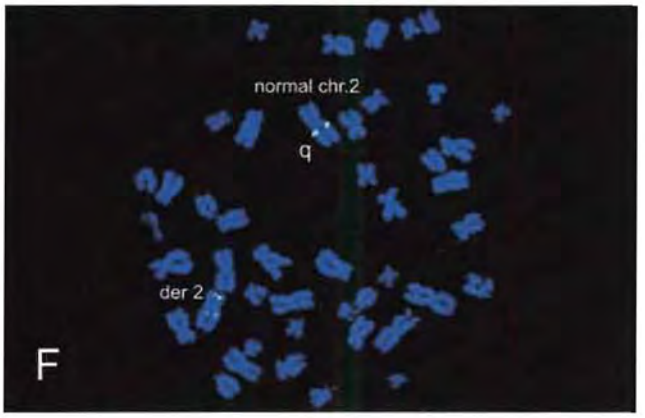
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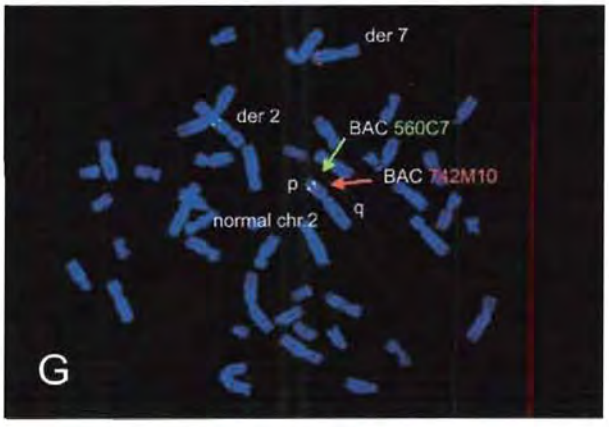
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To determine the precise breakpoints at the DNA level and identify possible genes interrupted by the breakpoints, further analysis by fluorescence in situ hybridization was undertaken.

Determination of involvement of chromosomes 2 and 7 by chromosomal paint and comparative genomic hybridization

As the cytogenetic rearrangement seemed very complex, and could not be resolved in detail by standard cytogenetic studies, a chromosomal paint with chromosomes 2 and 7 was performed on metaphase chromosomes of the affected individuals. This showed an insertion of part of chromosome 2 into chromosome 7q35–q36 of the father as well as of the children (Fig. 2A). To determine the region of chromosome 2 that was inserted into chromosome 7, we used the comparative genomic hybridization technique with DNA of the son. This showed a peak of the chromosome 2 region p21–p23 (Fig. 2B), indicating a trisomy for this region in the children. FISH analysis using telomere probes for chromosomes 2 and 7 showed that the telomeres were not involved in the rearrangement (data not shown).

Fine mapping of the involved regions using FISH analysis with BAC clones

Breakpoint on chromosome 7

The rearrangement was further resolved in more detail by FISH analysis using BAC clones that mapped to chromosomes 2 and 7. A number of BAC contigs were chosen from the National Center for Biotechnology Information (NCBI) in the chromosome 7q35–q36, 2p21–p23, and 2q21–q23 regions and BAC clones were selected to determine the different breakpoints. An overview of BACs crossing the breakpoints is indicated in Fig. 3. FISH analyses of these BACs are shown in Fig. 2. A breakpoint for the three patients was seen on chromosome 7q35–q36 using BAC 641E23 (Fig. 2C and black circle in Fig. 3A). It is clearly visible that the chromosome 7 sequence is interrupted by the chromosome 2p21–p23 insertion.

Breakpoints on chromosome 2

Deleted/inserted region 2p21–p23. The distal breakpoint of the chromosome 2p21–p23 region (white circle in Fig. 3A) could not be determined precisely due to a gap between two contigs [NT_005053 (distal contig) and NT_005204 (proximal contig)]. This breakpoint is located between the two BACs on either end of these contigs (BAC 596A4, the most distal clone on NT_005204, and BAC 398J17, the most proximal clone on NT_005053) or on the far end of one of these clones, which would make the result invisible with the FISH technique (see inset 1, Fig. 3B). The gap between these two BACs could be covered by designing primers on either end of each BAC (see Materials and methods) and by performing a PCR on genomic DNA. The resulting 1.6-kb PCR product was sequenced and contained the end sequences of both BACs contiguously, indicating that the BACs mentioned do not overlap but are perfectly connected. This sequence is represented in AC110925, a linear genomic fragment of 3994 bp. The proximal breakpoint of the chromosome 2p21–p23 region is represented by BAC 490M8 (Fig. 2D and blue circle in Fig. 3A).

Inverted region 2p23–q22. The breakpoints of the inversion are visualized in Figs. 2E and 2F. The upper breakpoint on the short arm of chromosome 2 is represented by BAC clone 342D1, localized on chromosome 2p23 (Fig. 2E and yellow circle in Fig. 3A) and the lower breakpoint on the long arm is represented by BAC clone 432O12, localized on chromosome 2q22 (Fig. 2F and red circle in Fig. 3A).

Complexity of the rearrangement

The inversion/deletion has not been a simple event, and the result is schematically shown in Fig. 3. The region of chromosome 2 (2p21–p23) that is deleted from the abnormal chromosome 2 was not located at the upper border of the inversion on chromosome 2, but somewhat farther down. This is shown by the fact that BAC 342D1 gives two signals on der2 and not one on chromosome 2p and one on chromosome 7q. In addition BAC clones distal to BAC 342D1 mapped to their normal position on the 2p arm of the abnormal chromosome. BAC clones localized proximal to BAC 342D1 mapped to the 2q arm of the abnormal chromosome 2. The order of the BAC clones has been confirmed with FISH experiments on metaphase chromosomes of the father, one of which is shown in Fig. 2G, using BAC clones 560C7 and 742M10 (little green circles in Fig. 3A). For the deletion to have taken place, two extra breaks must have occurred (white and blue circles in Fig. 3A). The green block is the chromosome 2p21–p23 region that is deleted from the abnormal chromosome 2 and inserted in chromosome 7q35–q36. The size of this block is estimated to be at least 12 Mb, based on the NCBI BAC contigs (build 29) that are now located in this region. This chromosomal region is present in triplicate in the affected children and 152 genes are placed within this region. Of these, 29 are confirmed genes, based on a clean alignment of mRNA (with or without EST evidence) to the genomic sequence. Of the remaining loci, 12 are based on EST evidence only, 35 are predicted with EST evidence, 26 are predicted only, 40 have some discrepancy between the mRNA sequence and the gene model, and for 10 there is no unambiguous solution as to what they should be. An imbalance in the expression of one or more genes in this region could explain the mental and growth retardation in the children (see Discussion).

The purple block is the upper border of the inverted region of chromosome 2. Based on the contig lengths given by NCBI we have estimated the size of this block to be around 6.5 Mb.

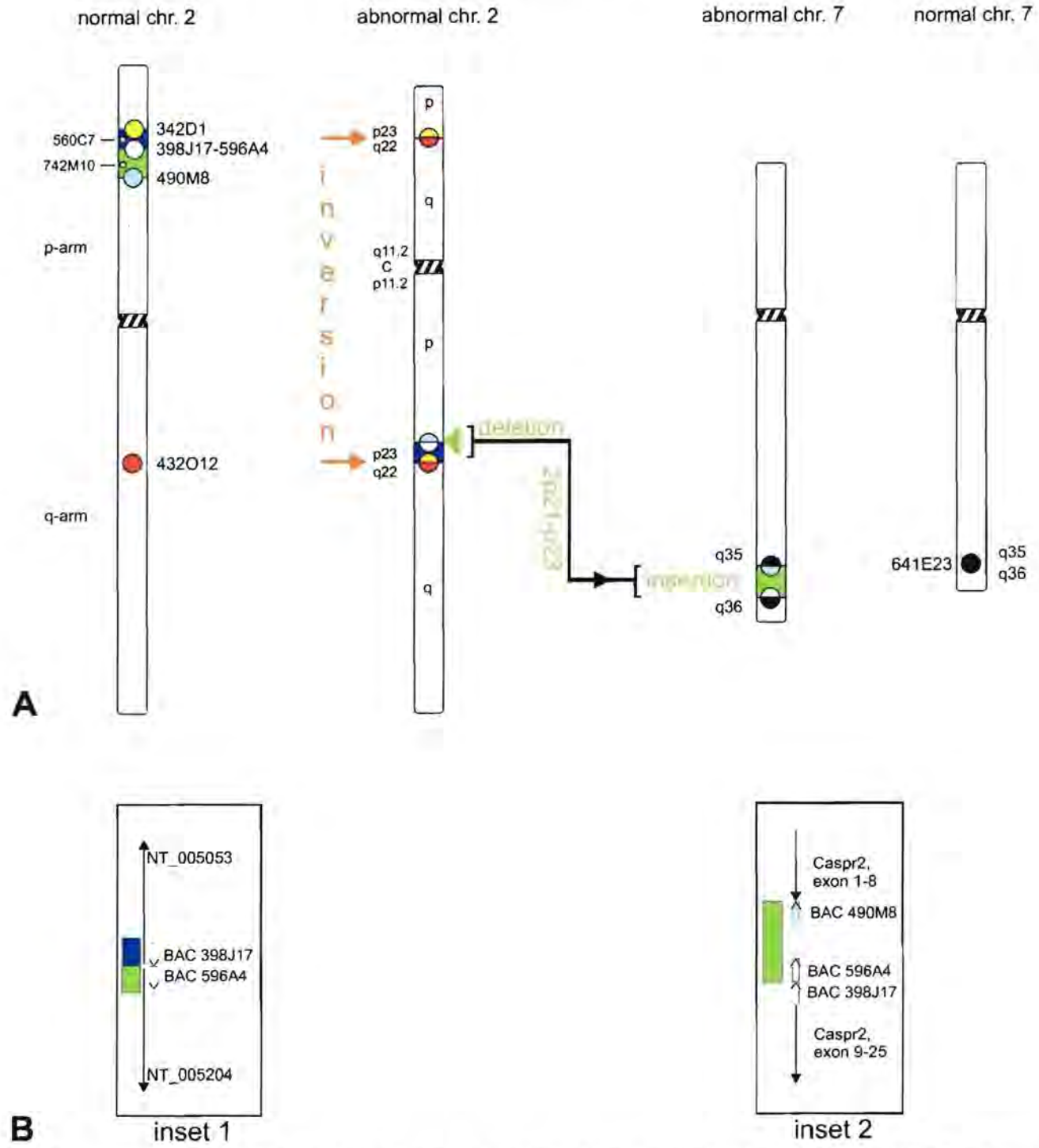

Fig. 3. Rearrangement as found in the father. BACs are indicated with colored circles. Breakpoints are indicated as half circles. The children have the same aberrant chromosome 7 and inherited two normal chromosomes 2. The green block is the chromosome 2p21–p23 region that is deleted in the abnormal chromosome 2 and inserted in chromosome 7q35–q36. The purple block is the upper border of the inversion. BAC 641E23 (black) is part of NCBI chromosome 7q contig NT_007194, BAC 342D1 (yellow) is part of NCBI chromosome 2p contig NT_0222275, BAC 490M8 (blue) is part of NCBI chromosome 2p contig NT_005367, BAC 432O12 (red) is part of NCBI chromosome 2q contig NT_022135, BAC 398J17 is the most proximal clone of contig NT_005053, and BAC 596A4 is the most distal clone on NT_005204. Insets are details of different breakpoints.

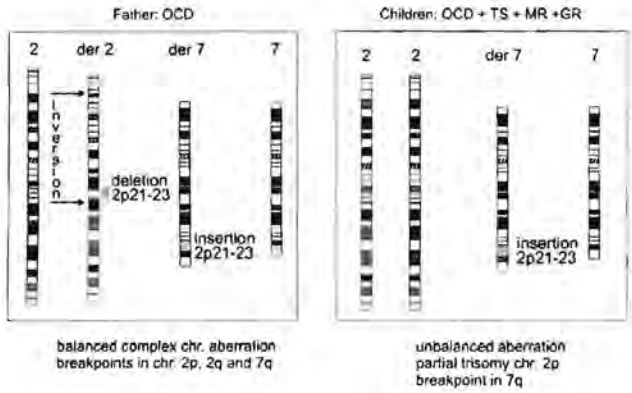

Fig. 4. Overview of the chromosomal rearrangements and clinical phenotype in the affected persons. OCD, obsessive compulsive disorder; TS, Tourette syndrome; MS, mental retardation; GR, growth retardation.

Contactin-associated protein 2

Because the common denominator in all three patients of the family is the breakpoint on chromosome 7q35–q36 with the chromosome 2p21–p23 insertion (Fig. 4), we initially concentrated on this region to search for interrupted genes. To find genes crossing the breakpoints, the BAC sequences were screened for genes using the NCBI Blast database. BAC 641E23, crossing the breakpoint on chromosome 7q35–q36 and similarly present in the three patients, showed homology to the contactin-associated protein 2 gene (*CNTNAP2*) (GenBank Accession No. AF19045). The *CNTNAP2* gene consists of 25 exons, has a genomic size of over 2.0 Mb [25], and is one of the largest genes in the human genome. It is predominantly expressed in the nervous system, at the juxtaparanodal region next to the nodes of Ranvier, and localized to the axonal membranes of large myelinated nerves [24]. CNTNAP2 is colocalized and associated with Shaker-type voltage-activated K^+ channels, which play a role in repolarization of the action potential [24,26].

Blast analysis with and alignment of the complete mRNA of *CNTNAP2* (AF319045, 8107 bp) showed the presence of exons 6, 7, and 8 on BAC 641E23. This was confirmed by PCR experiments, using primers in and around these exons on the BAC clone (data not shown). The orientation of *CNTNAP2* [25] was confirmed by FISH to be 5′ to 3′ from 7q centromere to 7q telomere, using different BAC clones containing exon 1 and exons 24 and 25 on the aberrant chromosome 7, so that clones could be distinguished above and below the chromosome 2 insertion (data not shown). Sequence data (NCBI, AC092676) showed that exons 6, 7, and 8 are present in the first 23 kb of the BAC clone 641E23. As the FISH signals of this BAC seem equally divided above and below the insertion on chromosome 7q35–q36 (Fig. 2) we concluded that the breakpoint must be localized in intron 8. Based on BAC sequence data from BAC clone 641E23 (containing exons 6, 7, and 8) and the overlapping BAC clone 643A21 (containing exon 9) the size of intron 8 is 167,632 bp.

Discussion

Chromosomal karyotypes in Gilles de la Tourette patients mostly are found to be normal [27], so in general there is no reason for standard chromosomal examination. When, however, in addition to GTS other clinical abnormalities are observed, chromosomal analysis is often called for. In the family described above, the father is the most mildly affected, with only obsessive–compulsive disorder; the children suffer from tics and OCD and in addition have growth and mental retardation. Comparing the chromosomal picture to the phenotype of the patients, the father has a balanced chromosomal abnormality. This includes an inversion of chromosome 2p23–q22 and a deletion of 2p21–p23, which has inserted into chromosome 7q35–q36, in addition creating a breakpoint in this region on chromosome 7. It is surprising that, despite this complex rearrangement, the only clinical feature in the father is OCD. The children each have an unbalanced situation with a partial trisomy for chromosome 2p21–p23 and the breakpoint on chromosome 7q35–q36. The children have OCD, GTS, and mental and growth retardation. For an overview, see Fig. 4.

A common feature in the three affected persons is the insertion of chromosome 2p21–p23 into chromosome 7q35–q36 with the breakpoints that accompany it. This suggests the presence of a gene for OCD/GTS on the breakpoint of chromosome 7q35–q36, which is shared by the three patients. The mental and growth retardation in the children could be accounted for by the presence of the trisomy of the chromosome 2p21–p23 region. The size of the chromosome 2 insertion into chromosome 7q35–q36 is estimated to be around 12 Mb, based on the current NCBI BAC contig data (build 29). An imbalance in the expression of one or more genes in this region could well account for the mental and growth retardation.

Partial trisomy 2p syndrome

Of interest in this regard are cases with rare partial trisomy 2p syndrome, involving different chromosomal regions between 2p12 and 2p24 [28]. Although in these patients the phenotype is variable, most likely due to different sizes of the trisomic region, mental retardation is present in all cases with involvement of 2p21–p24. Tourette-like symptoms have never been described in trisomy 2p syndrome patients, suggesting that Tourette symptoms are not caused by the trisomic region.

Possible fusion genes

In addition, there is also the possibility of interrupted genes on the chromosome 2 breakpoints. In principle only

the chromosome 2p21–p23 breakpoints of the insertion into chromosome 7q are of interest, as these are shared by the three patients. In this case, only fusion products of genes on chromosome 2 with one or both interrupted ends of *CNTNAP2* would be significant, possibly causing aberrant protein function. Therefor BACs 490M8, 397J17, and 596A4 were tested for the presence of genes by running them through the NIX analysis of the UK HGMP Resource Centre computing facilities and comparing with contig maps of the NCBI. Genes on BAC clones 398J17 and 596A4 would contain possible candidates to form fusion genes with the 3′ part of *CNTNAP2* (exon 9–25) (see insets 1 and 2, Fig. 3B). On BAC 398J17 three predicted genes were detected, of which only the hypothetical gene LOC130219 would be in the right orientation to be able to form a fusion product. On BAC 596A4 five predicted genes are placed, two of which would be in the same transcriptional orientation with CNTNAP: LOC165316, which is similar to mitochondrial capsule selenoprotein (a gene that is expressed in sperm [29]), and LOC165318, of which nothing is known. Genes on BAC clone 490M8 were investigated as possible candidates to form fusion genes with the 5′ part of *CNTNAP2* (exons 1–8) (inset 2, Fig. 3B). Two predicted genes are present on this BAC, the hypothetical gene (LOC130741) with unknown function and without conserved domains and one gene (LOC165443) that has similarity to 60S ribosomal protein L21 [30]. On the aberrant chromosome 7, either gene, when interrupted, would be in the same transcriptional orientation as *CNTNAP2*. For all possible fusion genes between genes present on the breakpoint BACs and *CNTNAP2*, at the moment, the effect would not be predictable.

CNTNAP2, potassium channels, and Tourette syndrome

Because of the insertion, and assuming that no functional fusion genes have formed, the *CNTNAP2* gene on chromosome 7q is interrupted on one copy. The implication of a lower expression of this gene during development is hard to predict, also because the function of this gene is not yet fully elucidated. CNTNAP2 belongs to the superfamily of the neurexins, a group of transmembrane proteins that mediate cell–cell interactions in the nervous system [24,31,32]. CNTNAP2 and CNTNAP1, another member of this family, are localized to the axonal nodes of Ranvier, but to different, distinct, compartments [24,31,33,34]. CNTNAP2 is located in the juxtaparanodal region and is associated with Shaker-like, voltage-gated, K^+ channels [24,34]. CNTNAP2 has a structure similar to that of CNTNAP1, containing various domains in the extracellular region, but they differ in their cytoplasmic tails [24,35].

Based on what is known of the movement of Na^+ channels during myelinogenesis and their subsequent clustering at each (para)node of Ranvier through the physical pushing activity of CNTNAP1 [34], it is speculated that CNTNAP2, possibly in association with other proteins, has a similar function in the localization of the K^+ channels to the juxtaparanode [24]. Tourette syndrome is a complex disorder, and its cause is unknown. It is hypothesized that symptoms of GTS are caused by the absence of inhibition of unwanted actions, movements as well as behaviors, regulated by the basal ganglia [36,37]. We hypothesize that disruption or decreased expression of CNTNAP2 could lead to a disturbed distribution of the juxtaparanodal Shaker-like K^+ channels in the nervous system, having an influence on conduction and/or the repolarization of the action potential in certain parts of the brain that are sensitive to these kinds of changes. Mutations in the K^+ channel in *Drosophila* leads to a hyperexcitable, leg-shaking phenotype (Shaker phenotype) [38]. In addition mutations in the Kv1.1 K^+ channel subunit are present in patients with episodic ataxia, a disease in which patients suffer from episodes of involuntary movements [39]. Deletion of Kv1.1 causes epilepsy in mice [40] and repetitive discharge from the nerve endings at neuromuscular junctions [41].

Future molecular studies like creating knockout mice for *CNTNAP2* or the K^+-channel subunits might shed some light on involvement of these genes in Tourette syndrome. In summary, we have identified a gene, *CNTNAP2*, that is interrupted in a family with Gilles de la Tourette syndrome/OCD. This gene is a candidate gene for GTS/OCD, as it is likely to be involved in signal transduction, a feature that is obviously disturbed in this disorder.

Materials and methods

Case reports

The *father* has no history of motor or vocal tics. He does have OCD, including some excessive handwashing, contamination fears, a need for strict timetables, and frequent checking and rewriting rituals. He also has evidence of recurrent major depressive disorder. The *mother* is unaffected, but stutters.

The *daughter* at age 5 years was found to have generalized developmental delay with specifically language delay, with an IQ of about 65, small stature, and microcephaly. She was noted to have inappropriate behaviors with other children, with unprovoked behaviors as well as ritualistic behaviors and significant perseveration. At the age of 8, she had multiple motor tics (which began at age 3) and vocal tics (which began around the age of 5) and continued to have ritualistic behaviors. At age 11 she continued to have motor and vocal tics as well as repetitive and ritualistic behaviors. She read at a second-grade level and wrote at a first-grade level.

The *son* was also found to be globally developmentally delayed, with small stature, esotropia, and a chronic dermatitis. At the age of 2 years and 2 months he had essentially no expressive language. At the age of 3½ he was found to have multiple motor tics but no vocal tics and had devel-

oped a sign language vocabulary of over 200 words. Like his sister he had multiple ritualistic behaviors, including a very involved bathing ritual. He was very perseverative about lining things up and tearing and cutting things, including paper, books, and clothes. At the age of 5 he spontaneously developed verbal language. IQ assessment gave him a full-scale IQ of 54. He was found to be reading at age level, although his verbal skills continue to be very low.

Clinical diagnosis of Tourette syndrome was made according to specific criteria described in the *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. [4,42]. Appropriate informed consent was obtained from human subjects.

Karyotyping

Karyotyping was done on metaphase chromosomes isolated from cultured blood samples and Epstein–Barr virus (EBV)-derived cell lines. The results were the same in both cases.

FISH analysis

FISH analyses were performed on metaphase chromosomes derived from EBV-transformed peripheral blood lymphocytes according to the protocols of Pinkel et al. [43] with minor modifications. Whole-chromosome-specific paints were obtained commercially (Eurodiagnostica). Probes were labeled with either biotin (Bio) or digoxigenin (Dig) using the Nick Labeling Kit (Roche). Bio/Dig were detected with a one-layer amplification step using streptavidin Alexa 594 (Molecular Probes) and anti-Dig-fluorescence (Roche). Chromosomes were counterstained with Vectashield (Vector) containing 4,6-diamidino-2-phenylindole (Sigma) (blue). Slides were analyzed with a Zeiss Axioskop II imaging fluorescence microscope and images were captured using a Genetiscan Power Gene System (Applied Imaging).

BAC clones

BAC clones were ordered from Dr. Pieter de Jong at BAC/PAC Resources (http://www.chori.org/BACPAC). All BACs (641E23, 342D1, 490M8, 432O12, 398J17, 596A4, 560C7, 742M10) were from the RPCI-11 library. For the isolation of DNA, BAC clones were grown overnight at 37°C in 350 ml LB medium with the relevant antibiotics. DNA was isolated using the Nucleobond AX Kit (Macherey–Nagel). Yields varied between 15 and 30 μg.

PCR

Primers used on genomic DNA to cover the gap between BACs 398J17 and 596A4 were 121E2-2R, 5′ GAGGCTTGGTGACCAGTATC 3′, and 121E2-1F, 5′ AGACGGAGATGTCCCATGAC 3′. Standard conditions were used, with 1.5 mM $MgCl_2$ and 35 cycles of 1.5 min at 55°C.

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NEURO IMMUNOLOGY

PAPER

Tourette's syndrome: a cross sectional study to examine the PANDAS hypothesis

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Background: The classical neurological disorder after group A β haemolytic streptococcal infection is Sydenham's chorea. Recently a tic disorder occurring after group A streptococcal infection has been described and termed PANDAS (paediatric autoimmune neuropsychiatric disorders associated with streptococcal infection). It is proposed that antibodies induced after group A streptococcal infection react with basal ganglia neurones in Sydenham's chorea and PANDAS. Anti-basal ganglia antibodies (ABGA) are present in most cases of acute Sydenham's chorea, but rarely in controls.
Objective: To investigate the hypothesis that Tourette's syndrome may be associated with group A streptococcal infection and ABGA.
Methods: 100 patients with Tourette's syndrome (DSM-IV-TR) were enrolled in a cross sectional study. Children with neurological disease (n = 50) and recent uncomplicated streptococcal infection (n = 40), adults with neurological disease (n = 50), and healthy adults (n = 50) were studied as controls. Recent group A streptococcal infection was defined using antistreptolysin O titre (ASOT). ABGA were detected using western immunoblotting and indirect immunofluorescence.
Results: ASOT was raised in 64% of children with Tourette's syndrome compared with 15% of paediatric neurological disease controls ($p < 0.0001$), and in 68% of adults with Tourette's syndrome compared with 12% of adult neurological controls and 8% of adult healthy controls ($p < 0.05$). Western immunoblotting showed positive binding in 20% of children and 27% of adults with Tourette's syndrome, compared with 2–4% of control groups ($p < 0.05$). The most common basal ganglia binding was to a 60 kDa antigen, similar to the proposed antigen in Sydenham's chorea. Indirect immunofluorescence revealed autoantibody binding to basal ganglia neurones. Serological evidence of recent group A streptococcal infection, assessed by a raised ASOT, was detected in 91% (21/23) of Tourette's syndrome patients with positive ABGA compared with 57% (44/77) with negative ABGA ($p < 0.01$).
Conclusions: The results support a role of group A streptococcal infection and basal ganglia autoimmunity in a subgroup of patients with Tourette's syndrome and suggest a pathogenic similarity between Sydenham's chorea and some patients with Tourette's syndrome.

Gilles de la Tourette's syndrome is characterised by multiple motor and vocal tics which wax and wane over time.[1] Epidemiological studies have shown that Tourette's syndrome is relatively common (occurring in up to 1% of children)[2–4] and is more prevalent in boys.[2 3] The mean age of onset is seven years.[1] Comorbid neuropsychiatric symptoms are a common feature, and include obsessive-compulsive disorder, attention deficit hyperactivity disorder (ADHD), anxiety, and depression. Indeed, Tourette's syndrome and obsessive-compulsive disorder may be part of the same disease spectrum.[1 3]

The results from large family studies have suggested that Tourette's syndrome is at least partly genetically determined, although no common single genetic locus has yet been demonstrated.[1] A multifactorial aetiology has therefore been proposed, with genetic predisposition and environmental factors (such as trauma and infection) playing roles in disease expression.[1] It is also conceivable that Tourette's syndrome is a heterogeneous disorder, which would confound genetic studies.

It has recently been suggested that group A β haemolytic streptococcal infections are an important factor in acute onset neuropsychiatric and movement disorders.[5] The classic post-streptococcal neurological disorder is Sydenham's chorea, which occurs weeks to months after group A streptococcal infection and is one of the major diagnostic criteria of rheumatic fever. In addition to chorea, patients with Sydenham's chorea also have characteristic behaviour disturbances, particularly emotional lability.[7] Follow up studies have also shown a high prevalence of obsessive-compulsive disorder in Sydenham's chorea.[8] Although half the patients with Sydenham's chorea have a self limiting illness, the remaining 50% will have a chronic course with relapses or persistence.[9]

Until the 1990s, Sydenham's chorea was considered to be the only neurological sequel of streptococcal infection. However, during an outbreak of group A streptococcal infection in Rhode Island, there were many reports of affected children developing sudden onset tics and psychiatric disorders.[10] Subsequently, the clinical phenotype of post-streptococcal tics and obsessive-compulsive disorder was described, and the term PANDAS (paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections) was coined.[6] Patients with PANDAS characteristically have exacerbations of characteristic symptoms after further group A streptococcal infections but otherwise have a clinical phenotype similar to Tourette's syndrome. This has led to the hypothesis that Tourette's syndrome may also be related to streptococcal infections, although this association remains

Abbreviations: ABGA, anti-basal ganglia antibodies; ADHD, attention deficit hyperactivity disorder; ASOT, antistreptolysin O titre; DSM, *Diagnostic and Statistical Manual of Mental Disorders*; ICD, *International Classification of Diseases*; PANDAS, paediatric autoimmune neuropsychiatric disorders associated with streptococcal infection

Table 1 Demographics of the sample

Group	n	Mean age (years) (range)	Sex (M/F)
Child Tourette syndrome	56	12.8 (8 to 17)	40/16
Adult Tourette syndrome	44	37.8 (18 to 61)	32/12
Child streptococcal infection	40	9.8 (2 to 15)	25/15
Child neurological disease	50	7.6 (0.5 to 18)	24/26
Adult healthy controls	50	35.6 (19 to 57)	25/25
Adult neurological disease controls	50	41.1 (19 to 70)	30/20

F, female; M, male.

controversial.[11] The proposed disease mechanism in Sydenham's chorea and PANDAS is cross reactive antibodies induced by group A streptococcal infection that bind specifically to basal ganglia antigens.[12] The presence of anti-basal ganglia antibodies (ABGA) in Sydenham's chorea[13–15] and PANDAS[10] supports this hypothesis.

We aimed to examine the association of streptococcal infection and basal ganglia autoimmunity in Tourette's syndrome by screening for recent streptococcal infection and ABGA in a large cohort of children and adults with this syndrome.

METHODS

Patients

Permission for the study was obtained from the local ethics committee of the National Hospital for Neurology and Neurosurgery. Index patients were interviewed by one of us (MMR) using standardised instruments including the National Hospital interview schedule,[16] the diagnostic interview schedule,[17] and the Yale global tic severity rating scale.[18] To be diagnosed as having Tourette's syndrome, patients had to satisfy DSM-IV-TR (APA, 2000) and ICD-10 (WHO, 1992) criteria. Thus they had to have multiple motor and one or more vocal tics, with symptoms lasting longer than a year. Comorbid diagnosis of obsessive-compulsive disorder and ADHD conformed to DSM-IV and ICD-10 criteria.

Controls

To determine the significance of both streptococcal serology and anti-basal ganglia antibodies, we enrolled controls for comparison (table 1), as follows:

- *Children with neurological disease* (n = 50). This group contained patients with dystonia (n = 30) of inflammatory (n = 12), vascular (n = 6), metabolic (n = 6), and genetic (n = 2) aetiology. Additional dystonic aetiologies included variant Creutzfeldt-Jakob disease, basal ganglia tumour, athetoid cerebral palsy, and juvenile Parkinson's disease (n = 1 each). Also within the neurology control group were patients with invasive viral encephalitis (n = 20).
- *Children with recent uncomplicated streptococcal infection* (n = 40).
- *Adults with neurological disease* (n = 50), including multiple sclerosis (n = 12), dementia (n = 7), paraneoplastic syndromes (n = 3), autoimmune neuropathy (n = 3), acute cerebellitis (n = 3), and other mixed neurological disease.
- *Healthy adults from laboratory staff and paediatric hospital workers* (n = 50).

The Tourette's syndrome patients and controls were recruited during the same time period and blood samples were stored at −80°C with identification data coded.

Streptococcal serology

Evidence of recent streptococcal infection was examined using antistreptolysin O titres (ASOT). All patient and control samples were analysed using standardised Dade Behring II nephelometry, an ASOT above 200 IU/ml is considered to indicate recent infection (WHO guidelines).

Basal ganglia homogenate

Caudate, putamen, and globus pallidus from a patient with no evidence of neurological disease was kindly provided by the Queen Square brain bank for neurological disorders, Institute of Neurology, London. The block of tissue was homogenised with a small volume of saline containing protease inhibitors (Sigma Chemicals, Poole, Dorset, UK) and centrifuged for 30 minutes at 7500 × *g*. Equal volumes of supernatant and Di-isopropyl ether were mixed and centrifuged at 600 × *g* for 10 minutes to remove lipid from the supernatant. The protein fraction was then collected and stored at −80°C until required.

Basal ganglia antibody western immunoblotting

As previously described[15] the basal ganglia homogenate was mixed with lithium dodecyl sulphate sample buffer (Invitrogen, USA), containing 0.05 M dithiothreitol and heated at 65°C for 15 minutes. A total of 30 µg of protein was loaded onto a 4–12% bis-tris gel (Invitrogen, Paisley, Scotland, UK) and electrophoresed. The proteins were blotted onto nitrocellulose (Sartorius, Epsom, Surrey, UK) and blocked with 2% milk proteins for two hours. Samples were diluted 1/300, applied to the blot, and incubated overnight at 4°C. The nitrocellulose was washed with 10 changes of 0.9% saline containing 0.2% milk proteins and 0.025% Tween. The blot was incubated for two hours with rabbit anti-human IgG conjugated with horseradish peroxide diluted 1/1000 (Dako, Cambridge, UK). After washing, the substrate 4-chloro-1-naphthol (Sigma) was added and the blot was allowed to develop for 15 minutes. Western immunoblotting was done in all patients and controls.

Basal ganglia antibody indirect immunofluorescence

Indirect immunofluorescence was undertaken in 10 western immunoblot positive Tourette's syndrome patients and 10 controls from each group. Using previously described methods,[11 15] 10 µm sections of normal human basal ganglia were cut from a snap frozen tissue block of caudate and putamen. Control and patient sera were diluted 1:10 in phosphate buffered saline (PBS), overlaid onto prepared slides, and incubated for 30 minutes. A control slide was also prepared and incubated with PBS alone for 30 minutes to assess background fluorescence. All slides were washed in PBS and incubated with anti-human IgG conjugated with fluorescein isothiocyanate (Dako) and analysed using a fluorescence microscope.

Statistical analysis

All statistical analyses were done using SAS software (SAS Institute Inc, Cary, North Carolina, USA). Streptococcal serology was compared using the non-parametric two sample exact Wilcoxon rank-sum test. Positive ABGA Western immunoblotting was compared in each subgroup using χ^2 tests. Ninety five per cent confidence intervals (CI) are given.

RESULTS

Demographics

Demographic data on the patients are summarised in table 1. The serology results were analysed in paediatric and adult

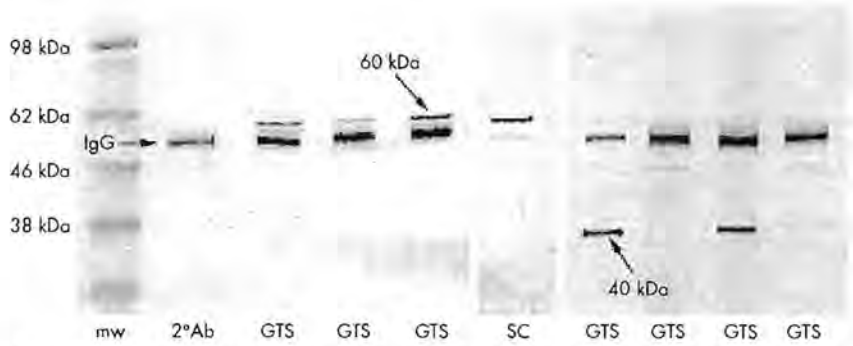

Figure 1 Western immunoblot against homogenate of human basal ganglia. An IgG band is present in lanes from both patients and controls and is from the human basal ganglia donor. Tourette syndrome patients have an IgG response against a 60 kDa basal ganglia antigen which is similar to the pattern seen in Sydenham's chorea. Other IgG binding is to a 40 kDa and a 45 kDa basal ganglia antigen. mw, molecular weight marker; GTS, Gilles de la Tourette syndrome patients; SC, Sydenham's chorea; 2°Ab, secondary antibody only.

groups separately, because of the higher incidence of streptococcal infection in childhood. The Tourette's syndrome cohort had a mean (SD) diagnostic confidence index of 64 (20)%, range 14% to 100%. The prevalences of obsessive-compulsive disorder and ADHD in the total cohort were 31% and 43%, respectively. The paediatric Tourette's syndrome patients had a diagnostic confidence index of 64 (21)%, range 14% to 100%, a 17% incidence of obsessive-compulsive disorder, and a 49% incidence of ADHD. The adult Tourette's syndrome patients had a diagnostic confidence index of 63 (20)%, range 16% to 100%, a 51% incidence of obsessive-compulsive disorder, and a 31% incidence of ADHD in childhood.

Streptococcal serology

Paediatric

As predicted, ASOT was raised in 80% of the children with recent streptococcal infection (mean 349 IU/ml (95% CI, 270 to 427)). ASOT was also raised in 64% of the children with Tourette's syndrome (299 IU/ml (262 to 335)) and in 18% of the children with neurological disease (151 IU/ml (101 to 201)). ASOT was raised in the paediatric Tourette's syndrome cohort compared with the neurological controls ($p < 0.0001$), but not with the streptococcal controls ($p = 0.4$).

Adult

ASOT was raised in 68% of the adults with Tourette's syndrome (mean 298 IU/ml (95% CI, 243 to 353), in 12% of the adults with neurological disease (140 IU/ml (92 to 188)), and in 8% of the healthy adults (122 IU/ml (101 to 143)). ASOT was statistically raised in the adult Tourette's syndrome cohort compared with the neurological controls ($p < 0.05$) and the healthy controls ($p < 0.05$).

ABGA western immunoblotting

Paediatric

Twenty per cent of the paediatric Tourette's syndrome group (12/56) had positive western immunoblotting, compared with 4% of the neurological controls (1/50) and 2% of the streptococcal controls (1/40). The difference between the Tourette's syndrome group and both control groups was significant ($p < 0.05$ and $p < 0.05$, respectively). Common bands of reactivity were seen in the Tourette's syndrome group, rather than polyspecific binding. The most common responses were to 60 kDa (n = 6), 40 kDa (n = 4), 45 kDa (n = 3), 67 kDa (n = 2), 80 kDa (n = 2), and 95 kDa antigens (n = 1) (fig 1). The positive neurological controls bound to a 40 kDa (n = 1) and 43 kDa (n = 1) antigen. The positive streptococcal control bound to a 60 kDa antigen.

Adult

Twenty seven per cent of the adult Tourette's syndrome group (12/44) had positive western immunoblotting, compared with 2% of the neurological controls (1/50) and 4% of the healthy controls (2/50) ($p < 0.005$ and $p < 0.005$, respectively). The common antibody binding was also to 60 kDa (n = 7), 40 kDa (n = 5), 45 kDa (n = 2), 80 kDa (n = 2), 67 kDa (n = 1), and 98 kDa antigens (n = 1). The positive neurological control bound to a 40 kDa antigen. The positive healthy controls both bound to a 55 kDa antigen. In all paediatric and adult Tourette's syndrome patients the most common basal ganglia autoantigen detected was to a 60 kDa protein (n = 13) and then to a 40 kDa protein (n = 9).

ABGA indirect immunofluorescence

To define the localisation of antibody binding, we carried out indirect immunofluorescence on 10 Tourette's syndrome patients (five paediatric and five adult) with positive western immunoblotting. All patients had the same binding pattern, with IgG binding to large basal ganglia neurones (fig 2). The

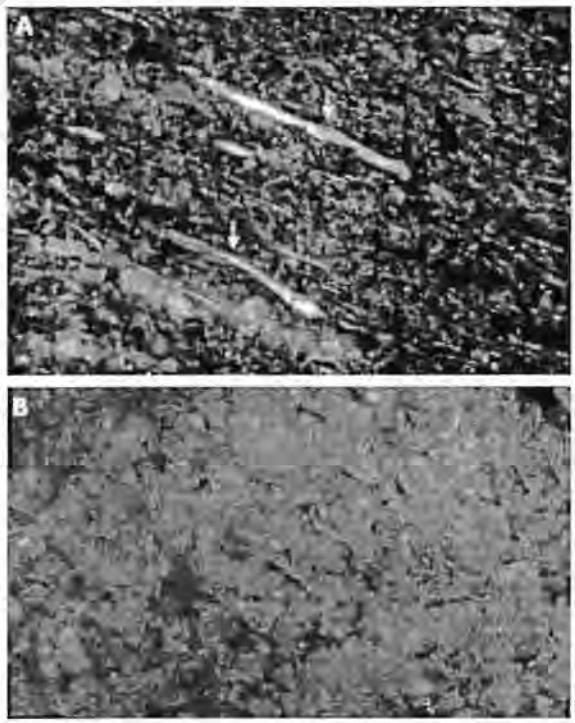

Figure 2 (A) Basal ganglia immunofluorescence revealing antibody binding to basal ganglia neurones in a patient with Tourette's syndrome. (B) Negative binding in control.

Table 2 Clinical comparisons between ABGA positive and ABGA negative Tourette's syndrome patients*

Finding	ABGA positive (n=24)	ABGA negative (n=77)	p Value
Age at first tic (years) (range)	6.56 (2 to 13)	6.81 (2 to 16)	NS
Diagnostic confidence index (mean (SD), [range])	62 (20)% [36% to 96%]	64 (21)% [14% to 100%]	NS
Obsessive-compulsive behaviour disorder	27%	33%	NS
Attention deficit hyperactivity disorder	22%	50%	<0.05
History of neuropsychiatric disease in 1st degree family member†	69.6%	753%	NS
ASOT >200 IU/ml	91.3%	584%	<0.01

*By western immunoblotting.
†Family history of neuropsychiatric disease includes tic disorders, Tourette's syndrome, and obsessive-compulsive disorder.
ABGA, anti-basal ganglia antibodies; ASOT, antistreptolysin O titre.

Table 3 Follow up studies

Case	Test	1st Test result	2nd Test result	Time after 1st test (months)
Case 1	ASOT	500 IU/ml	133 IU/ml	3
	ABGA	Negative	60 kDa	
Case 2	ASOT	260 IU/ml	185 IU/ml	3.5
	ABGA	Negative	45 and 60 kDa	
Case 3	ASOT	710 IU/ml	220 IU/ml	4
	ABGA	Negative	40 kDa	
Case 4	ASOT	315 IU/ml	<50 IU/ml	3
	ABGA	Negative	40 and 95 kDa	

ABGA, anti-basal ganglia antibodies; ASOT, antistreptolysin O titre.

immunofluorescence staining pattern seen using these Tourette's syndrome patients was identical to that previously described in Sydenham's chorea.[15] None of the controls tested had reactivity against any cellular component of the basal ganglia.

Raised streptococcal serology and positive ABGA

Ninety one per cent of Tourette's syndrome patients with positive ABGA western immunoblotting (21/23) had a positive ASOT, compared with 57% of Tourette's syndrome patients with negative ABGA western immunoblotting (44/77) ($p < 0.01$).

Clinical comparison between ABGA positive and ABGA negative groups

The clinical characteristics (obsessive-compulsive disorder/ADHD and family history of neuropsychiatric disease) did not differ between patients who were ABGA positive or ABGA negative (table 2). The numbers of patients with a significantly raised ASOT were greater in the ABGA positive group (91.3%) than in the ABGA negative group (58.4%). We were able to retest the blood of four of the Tourette's syndrome patients who had negative ABGA and positive ASOT 3.5 months after first testing. This showed that ASOT levels had decreased to normal in three cases but ABGA had become positive in all four (table 3).

DISCUSSION

The Tourette's syndrome patients were recruited from a dedicated clinic with all patients fulfilling strict diagnostic criteria. The mean age of onset (6.75 years) and the male predominance were consistent with previously published data.[1 3] Similarly, 100% of the patients in this study presented in childhood (< 18 years), which is characteristic of Tourette's syndrome.[1 3] As longitudinal studies have shown that 50% of patients will be free of tics by 18 years of age, and tic severity gradually diminishes in adulthood,[1] it is likely that this cohort of adult patients with Tourette's syndrome represents a severe form of the disease.

The pathogenesis of Tourette's syndrome remains obscure but it is considered to be an inherited neurodevelopmental disorder resulting in disinhibition of the cortico-striatal-thalamic-cortical circuitry.[1] Although various neurotransmitters have been implicated in the pathogenesis of the syndrome, the most favoured hypothesis is that there are subtle abnormalities of the dopaminergic system.[19] Tourette's syndrome appears to be a familial disorder, with early reports supporting an autosomal dominant inheritance.[20 21] A systematic whole genome screen revealed two regions, 4q and 8p, with increased lod scores, and these loci might yet reveal susceptibility genes for Tourette's syndrome.[22] As a genetic basis for Tourette's syndrome has yet to be uncovered, alternative pathological models are being considered, and Tourette's syndrome may turn out to be a heterogeneous disorder.[23]

The recognition that PANDAS may be pathologically related to Sydenham's chorea, along with the clinical similarity of PANDAS and Tourette's syndrome, has led to the notion that Tourette's syndrome may occur as a result of basal ganglia dysfunction secondary to post-streptococcal autoimmunity.[24] The observation that patients with tic disorders or Tourette's syndrome are more likely to have positive streptococcal serology than control subjects[25–27] supports this hypothesis, though not all studies have replicated such an association.[28 29] Preliminary analysis of antibody reactivity to streptococcal M proteins has shown raised titres to M12 and M19—but not to M1, M4, and M6—in 25 adult patients with Tourette's

syndrome compared with 25 control subjects.[26] This may suggest that only certain strains of group A streptococcal infection could be linked to Tourette's syndrome. This is compatible with observations that other post-streptococcal immune mediated disorders, such as acute rheumatic fever and glomerulonephritis, are also strain specific.[30]

The proposal that Sydenham's chorea and PANDAS occur as the result of an immune mediated insult is supported by the presence of ABGA which bind to basal ganglia neurones.[12–14] In a study of patients with Sydenham's chorea, we have recently shown that ABGA were present in all 20 patients (100%) with acute disease and in 11/16 patients (69%) with persistent disease, but in none of a group of 11 healthy controls.[15] Increased antibody binding to putamen in Tourette's syndrome, and identification of basal ganglia antigens of molecular weights 60, 67, and 83 kDa, have been reported.[28] A further study also suggested that 60 kDa was the most prevalent basal ganglia autoantigen in Tourette's syndrome, although a complex multivariate analysis is required to establish this association.[31] Preliminary results from another group also suggested that a 60 kDa and an 83 kDa antigen represented the dominant response in patients with tics, Tourette's syndrome, or obsessive-compulsive disorder,[32] and the same investigators suggested that these antibodies recognised a calpain–calpastatin complex.[33] In Sydenham's chorea, we have described three dominant basal ganglia autoantigens of molecular weights 40, 45, and 60 kDa.[15] The consistent finding of a 60 kDa antigen from this study may suggest that the antigen could be important in Sydenham's chorea, PANDAS, and Tourette's syndrome.

Not all of the studies reported have had similar findings to ours. Two studies using a neuroblastoma cell line rather than basal ganglia as the antigen source found no discriminate antibody responses in Tourette's syndrome.[27 31] It would appear from these studies that human or mammalian brain homogenates should be the preferential source of antigen. The methods for detecting antibodies may also influence the results from different studies; enhanced chemiluminescence is a very sensitive method but produces a multitude of responses in western immunoblotting of patients and controls, most of which are of doubtful significance.[27–29 31] We propose that colorimetric analysis of western blots in this setting is preferable[15] and improves specificity, although arguably at the cost of reduced sensitivity.

Using the methods described we found a correlation between ABGA and positive ASOT. Serological evidence of recent group A streptococcal infection was detected in 91% of patients with positive ABGA and in 57% with negative ABGA. This may suggest that ABGA and recent streptococcal infection are related in some patients. Interestingly the prevalence of raised ASOT in the ABGA negative patients was significantly more common than in the control groups. This finding suggests that post-streptococcal autoimmunity could be important in a larger proportion of patients with Tourette's syndrome. It is possible that ABGA reactivity is be a phenomenon that waxes and wanes with the clinical course. Indeed three of four patients whom we followed up with positive ASOT but negative ABGA had positive ABGA and decreased ASOT three months after the initial sample was taken. A longitudinal study is required to investigate the temporal association between ABGA, streptococcal serology, and clinical course.

An alternative explanation for ABGA in Sydenham's chorea, PANDAS, and Tourette's syndrome is that it occurs as an epiphenomenonon secondary to basal ganglia damage. We believe this is unlikely, for three reasons. First, control patients with inflammatory, metabolic, and ischaemic basal ganglia diseases uncommonly develop these antibodies. Second, in a provocative study by Hallett and coworkers, the microinfusion of sera or IgG from patients with Tourette's syndrome into the striatum of rats induced stereotypes analogous to the involuntary movements seen in Tourette's syndrome,[34] suggesting that ABGA may play a pathogenic role. Finally, a randomised trial of therapeutic plasma exchange and intravenous immunoglobulin for PANDAS showed a therapeutic response in favour of the treated patients.[35]

Conclusions

We have shown that a significant proportion of patients with Tourette's syndrome have evidence of recent streptococcal infection and anti-basal ganglia antibodies. The studies published so far on these antibodies have used immunofluorescence, western immunoblotting, and enzyme linked immunosorbent assay methods, which have different sensitivities and specificities. This may help explain the differences between studies in the reported associations between ABGA and disease. Many investigators have proposed that the presence a 60 kDa autoantigen in Sydenham's chorea, PANDAS, and Tourette's syndrome is significant. The identification of this antigen is crucial in determining the pathogenicity of ABGA in post-streptococcal CNS syndromes.

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Soluble adhesion molecules in Gilles de la Tourette's syndrome

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Abstract

To investigate the immune-mediated response in TS, and its relationship with streptococcal infection, we measured serum levels of soluble intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin in patients with TS, compared to healthy and diseased controls. Soluble VCAM-1 and sE-selectin were significantly elevated in children and adults with TS, and sVCAM-1 was higher among anti-basal ganglia antibodies (ABGA)-positive adults with TS. No correlation of adhesion molecule levels to clinical severity or anti-streptococcal antibodies was observed. Children with Sydenham's chorea and paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) showed an increased level of sICAM-1, but not sVCAM-1 and sE-selectin. These results provide initial evidence for a role of adhesion molecules and systemic inflammation in TS, and support the hypothesis of an ongoing immune-mediated process in this condition.

Keywords: Tourette's syndrome; sICAM-1; sVCAM-1; sE-selectin; Adhesion molecules; Streptococcal infection; Anti-basal ganglia antibodies

1. Introduction

Gilles de la Tourette's syndrome (TS) is characterised by the presence of multiple chronic motor and phonic tics [1]. The aetiology of TS is still undefined, although several different genetic and environmental factors might play a role [2–5]. A relationship between the occurrence of tic disorders and Group A β-hemolytic streptococcal (GABHS) infections has been proposed [4] and intensely debated [6–8], leading to the description of the PANDAS (Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections) syndrome [9]. Interestingly, PANDAS show similarities to TS, mainly the predominance of tics in both syndromes, a high frequency of psychiatric comorbidity, such as obsessive–compulsive disorder (OCD) and anxiety disorders, and the waxing and waning of symptoms [1,9,10].

On the model of Sydenham's chorea (SC), a neuropsychiatric disorder related to rheumatic fever (RF), PANDAS are hypothesized to be an autoaggressive disorder triggered by molecular mimicry between surface GABHS antigens and neuronal antigens, enriched in the basal ganglia [11,12]. Cross-reacting autoantibodies were proposed to play a role in this process [11,13], and serum anti-basal ganglia antibodies (ABGA) were suggested as a potential diagnostic marker in post-streptococcal neurological and psychiatric disorders [14,15].

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Several reports showed elevated titers of anti-streptococcal antibodies in TS [16–18], and a cross-sectional study reported a higher frequency of ABGA in the serum of 100 patients with TS, compared to healthy and diseased controls [18]. This preliminary evidence led to the hypothesis that autoimmunity directed against the basal ganglia, triggered by GABHS infection, is involved in a subgroup of patients with TS. However, reports on markers related to systemic autoimmune diseases have been rare in TS.

Cellular adhesion molecules (intercellular adhesion molecule-1, ICAM-1; vascular cell adhesion molecule-1, VCAM-1; selectins) are glycoproteins belonging to the immunoglobulin superfamily, which mediate cell–cell and cell–extracellular matrix interactions [19]. Their expression is increased in a large number of inflammatory and immune-mediated conditions, including multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE) [20]. When expressed by endothelial cells in their membrane-bound form, these molecules act as ligands for leukocytes to facilitate their entry into the sites of inflammation [19]. Serum levels of soluble ICAM-1 are raised also in patients with RF, and such increase is maintained over time, until clinical remission [21].

To explore the concurrence of an immune-mediated inflammatory response in TS, we measured serum levels of the soluble forms of three adhesion molecules in children and adults with TS, and analysed their relationship to serological markers of recent streptococcal infection and the presence of ABGA.

2. Materials and methods

2.1. Subjects

Permission for the study was obtained from the ethics committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology. Thirty-three children and 51 adults diagnosed with TS were consecutively recruited from a tertiary referral centre of the same institution. Patients diagnosed with TS fulfilled DSM-IV-TR (APA, 2000) and ICD-10 (WHO, 1992) criteria, and were not preselected on the basis of a preceding streptococcal infection. Diagnostic interviews were performed by two specialists in TS (MO and MMR), using standardised instruments, including the National Hospital interview schedule [22], the Diagnostic Confidence Index (DCI) [23] and the Yale global tic severity rating scale (YGTSS) [24].

To determine the significance of serum levels of soluble ICAM-1 (sICAM-1), soluble VCAM-1 (sVCAM-1) and soluble E-selectin (sE-selectin), we studied several controls for comparison:

Children with non-inflammatory neurological diseases (NNID, $n=35$). This group contained 10 children with developmental delay, 14 children with epilepsy and 11 children with diagnosed CNS metabolic disorders.

Healthy children ($n=34$).

Children with post-streptococcal neuropsychiatric diseases (SC or PANDAS, $n=19$). Nine patients were diagnosed with acute SC: they all presented with an acute onset of chorea, met the modified Jones criteria for RF [25], and other causes of chorea were excluded. Ten patients fulfilled the working criteria for PANDAS [9]. All children in this group entered the study within 2 weeks from the onset of the acute illness.

Adults with non-inflammatory neurological diseases (NNID, $n=40$). This group contained 28 patients with primary adult-onset dystonia, 7 patients with primary hemifacial spasm, 3 patients with dopa-responsive dystonia, 1 with paroxysmal kinesigenic choreoathetosis and 1 with DYT-1 dystonia.

Healthy adults ($n=30$).

Subjects with NNID and children with SC or PANDAS were consecutively recruited from other tertiary referral centres of the same institution. Healthy controls were recruited from laboratory staff and hospital workers, and from their relatives. Diseased and healthy controls were matched to TS patients by age (±5 years), recruiting each suitable subject in a consecutive fashion. No subject, other than SC/PANDAS patients, had had RF, SC or an autoimmune disease. Case or control subjects affected by a clinically evident infectious or inflammatory disorder at the time of recruitment were excluded from the study. All subjects were recruited during the same period. A blood sample was taken from each subject, and aliquots of serum specimens were frozen within 30 min of collection and stored at −80 °C with identification data coded until analysis.

2.2. Adhesion molecules

Soluble adhesion molecules (sICAM-1, sVCAM-1 and sE-selectin) were quantified from patient sera using a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems Europe, Abingdon, Oxon, UK), following the protocol of the manufacturer. A monoclonal antibody specific for sICAM-1 had been pre-coated onto a 96 well microplate. Antibody to recombinant human sICAM-1 conjugated to horseradish peroxidase was then added to the wells. Standards, positive controls and sera were then incubated for 90 min at room temperature, at a 1:20 dilution. After washing, a colorimetric reading of the microplate by measurement of the absorbance at 450 nm was performed, using a tetramethylbenzidine solution as substrate. The same protocol was used for sVCAM-1 and sE-selectin quantitative measurements, using a 1:50 dilution for sVCAM-1 and a 1:20 dilution for sE-selectin.

2.3. Streptococcal serology

Evidence of recent streptococcal infection was examined using antistreptolysin O (ASOT) and anti-DNAse B titers.

For the ASOT measurements, samples were analysed using standardised Dade Behring II nephelometry; an ASOT above 200 IU/ml was considered to indicate recent infection (WHO guidelines). For the anti-DNAse B measurements, samples were analysed by a semiquantitative neutralising assay (Ph Plate, Stockholm, Sweden). The assay measures neutralisation of streptococcal DNAse enzyme B by colorimetric visual reading, using DNA–methyl green as the substrate; an anti-DNAse B titer above 300 IU/ml was considered to indicate recent infection.

2.4. Anti-basal ganglia antibodies in patients with TS

The Western immunoblotting method to detect ABGA has been previously described [26]. The antigen used was derived from human basal ganglia (caudate and putamen) and came from a donor with no history or evidence of neurological disease. The basal ganglia homogenate was mixed with lithium dodecyl sulphate sample buffer (Invitrogen, USA), containing 0.05 M dithiothreitol and heated at 65 °C for 15 min. A total of 30 ig of protein was loaded onto a 4–12% Bis–Tris gel (Invitrogen, Paisley, Scotland, UK) and electrophoresed. The proteins were blotted onto nitrocellulose (Sartorius, Epsom, Surrey, UK) and blocked with 2% milk proteins for 2 h. Samples were diluted 1/300, applied to the blot and incubated overnight at 4 °C. The nitrocellulose was washed with 10 changes of 0.9% saline containing 0.2% milk proteins and 0.025% Tween. The blot was incubated for 2 h with rabbit anti-human IgG conjugated with horseradish peroxidase diluted 1/1000 (Dako, Cambridge, UK). After washing, the substrate 4-chloro-1-naphthol (Sigma) was added and the blot was allowed to develop for 15 min. The test was considered positive when reactivity to at least one of three previously reported [26] common basal ganglia antigens (40, 45 and 60 kDa) was detected.

2.5. Statistics

SPSS (SPSS Inc., U.S.A.) was used for all statistical analyses. Serum soluble adhesion molecules levels from the different groups were compared using a one-way analysis of variance (ANOVA), when data from the compared groups passed the normality assumption test, or a Kruskal–Wallis one-way ANOVA, when data did not pass the normality assumption test or the difference between their standard deviation (SD) was significant. Assumption of normality was tested using the method of Kolmogorov and Smirnov. Scheffe test was used for multiple comparisons between groups. Correlation between soluble adhesion molecule values and ASOT values, YTSS score and DCI, expressed as continuous variables, was assessed by logistic linear regression analysis. ASOT values, YTSS and DCI scores were further categorized in a binomial fashion (0/1 scores), in the following ways: lower than/higher or equal to 200 IU for ASOT, lower than/higher or equal to median value for YTSS and DCI. Due to the semiquantitative type of determination, anti-DNAse B antibody titer was analysed only as a binomially categorized variable. Subsequently, frequency distribution of elevated soluble adhesion molecules values and ASOT (normal or elevated), anti-DNAse B (normal or elevated), YTSS (0 or 1), DCI (0 or 1) and ABGA status (negative or positive), was assessed by Fisher's Exact Test, categorizing the soluble adhesion molecules values of patients in lower than/higher or equal to the mean value of the healthy+2 S.D. Differences between proportions were assessed using two-sample t-test for proportions.

3. Results

3.1. Demographics

Patients' demographic data are summarised in Table 1. The mean (S.D.) duration of disease in patients with TS was 6.04 (2.5) years in children and 24 (7.2) years in adults. The median (25th–75th percentile range, rounded to the nearest integer) value of DCI was 55 (46–74) in children and 62 (55–75) in adults. The median (25th–75th percentile range) value of YTSS was 47 (23–56) in children and 49 (39–63) in adults.

3.2. Soluble adhesion molecules

Fig. 1 presents the distribution of adhesion molecules levels in the different groups, p values for differences between groups using the one-way ANOVA in all groups and the results of Scheffe test for multiple comparisons between groups.

3.3. Correlation between clinical scores and soluble adhesion molecules

In both paediatric and adult groups of TS patients, soluble adhesion molecule levels did not correlate to age and duration of disease ($p>0.05$ for all linear regression analyses). In the adult TS group, only sE-selectin values positively correlated to DCI ($r=0.56$; $p=0.04$). In both paediatric and adult TS groups, sICAM-1 and sVCAM-1 values did not significantly correlate to YTSS and DCI ($p>0.05$ for all correlation analyses, all Fisher's Exact

Table 1
Demographic features of participating subjects

	Age mean (range)	Sex (M/F)
Children with TS	12.8 (4–17)	26/7
Children with NNID	8.6 (4–17)	23/12
Healthy children	9.6 (3–16)	24/10
Children with SC/PANDAS	11 (6–22)	11/8
Adults with TS	32.9 (18–66)	39/16
Adults with NNID	38.5 (19–56)	15/25
Healthy adults	36.8 (18–69)	20/10

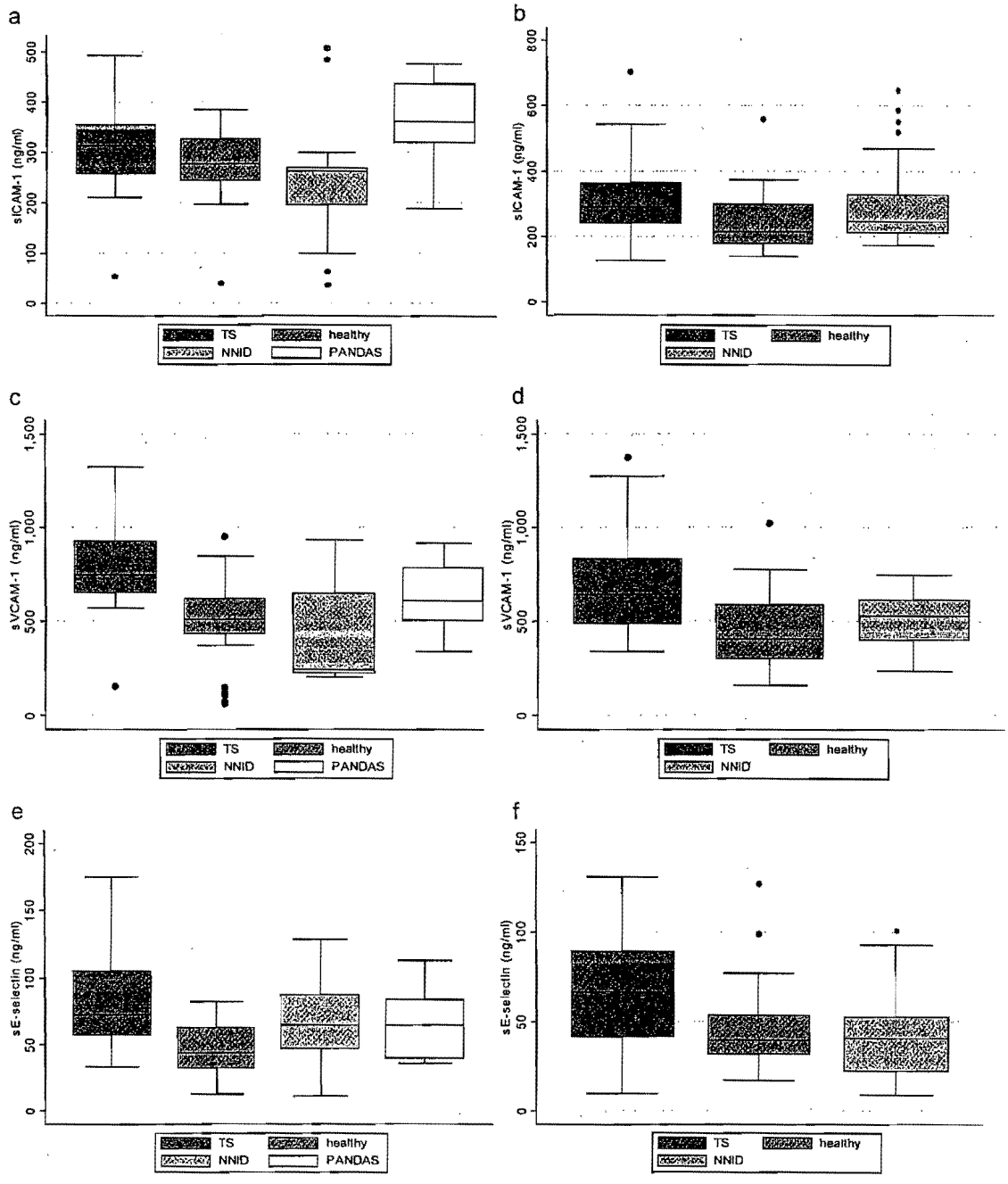

Fig. 1. (a) Soluble ICAM-1 levels in paediatric groups. One-way ANOVA: $F=6.51$, $p=0.0004$. Scheffe test for multiple comparisons between groups: TS>healthy, NS; TS>NNID, NS; SC/PANDAS>TS, NS; SC/PANDAS>healthy, $p=0.01$; SC/PANDAS>NNID, $p=0.02$. (b) Soluble ICAM-1 levels in adult groups. One-way ANOVA: $F=2.37$, $p=0.09$. Scheffe test for multiple comparisons between groups: TS>healthy, NS; TS>NNID, NS. (c) Soluble VCAM-1 levels in paediatric groups. One-way ANOVA: $F=4.76$, $p=0.004$. Scheffe test for multiple comparisons between groups: TS>healthy, $p=0.017$; TS>NNID, $p=0.016$; TS>SC/PANDAS, NS; SC/PANDAS>healthy, NS; SC/PANDAS>NNID, NS. (d) Soluble VCAM-1 levels in adult groups. One-way ANOVA: $F=15.62$, $p<0.0001$. Scheffe test for multiple comparisons between groups: TS>healthy, $p<0.001$; TS>NNID, $p=0.001$. (e) Soluble E-selectin levels in paediatric groups. One-way ANOVA: $F=8.8$, $p<0.0001$. Scheffe test for multiple comparisons between groups: TS>healthy, $p<0.0001$; TS>NNID, NS; TS>SC/PANDAS, NS; SC/PANDAS>healthy, NS; SC/PANDAS>NNID, NS. (f) Soluble E-selectin levels in adult groups. One-way ANOVA: $F=5.87$, $p=0.004$. Scheffe test for multiple comparisons between groups: TS>healthy, $p=0.034$; TS>NNID, $p=0.011$.

Tests>0.05), when analysed both as continuous and binary variables. Likewise, serum sE-selectin levels did not correlate to YTSS score in children and in adults with TS, when analysed both as continuous and binary variables ($p>0.05$ for all correlation analyses, all Fisher's Exact Tests>0.05).

3.4. Correlation and frequency distribution of streptococcal serology and soluble adhesion molecules

ASOT was raised in 23 (69%) of the 33 children with TS, and in 33 (65%) of the 51 adults with TS. In both paediatric and adult groups with TS, there was no significant

association between serum levels of the three adhesion molecules and ASOT, when analysed both as continuous ($p>0.05$ for all linear regression analyses) and binary (all Fisher's Exact Tests>0.05) variables.

Anti-DNAse B antibody titers were raised in 10 (30.3%) of the 33 children with TS, and in 9 (17.6%) of the 51 adults with TS. When compared to children with TS, anti-DNAse B antibody titers were significantly lower in healthy children (4/34 positive results, 11.8%, $p=0.03$), whereas there was no significant difference between TS children and paediatric non-inflammatory neurological controls (8/35 positive results, 22.6%, $p=0.23$). When compared to TS adults, anti-DNAse B antibody titers were significantly lower in healthy adults (0/30 positive results, $p=0.01$) and in adult non-inflammatory neurological controls (1/40 positive results, $p=0.02$).

In both paediatric and adult groups with TS, there was no significant association between serum levels of the three adhesion molecules and anti-DNAse B antibody titers (all Fisher's Exact Tests>0.05).

3.5. Frequency distribution of ABGA and soluble adhesion molecules

ABGA against three previously reported common basal ganglia antigens (40, 45, 60 kDa) were detected by Western immunoblotting in 10 (30%) of the 33 children with TS, and in 15 (29%) of the 51 adults with TS. There was no significant association between elevated sICAM-1 serum level and ABGA, both in children (Fisher's Exact Test=0.07) and in adults (Fisher's Exact Test=0.76). Elevated serum levels of sVCAM-1 were associated with ABGA in adults with TS (Fisher's Exact Test=0.046), whereas there was no association in children (Fisher's Exact Test=1.000). There was no significant association between elevated sE-selectin serum levels and ABGA, both in children and in adults with TS (Fisher's Exact Test=0.46 and 1.000, respectively).

4. Discussion

We report significantly increased titers of sVCAM-1 and sE-selectin in the serum of children and adults with TS, compared to healthy and NNID controls; this increase was less striking for sE-selectin in children, in whom there was no significant difference between patients and NNID controls. Conversely, sICAM-1 levels did not differ between patients with TS and controls. Children with SC/PANDAS had elevated titers of sICAM-1, when compared to healthy and NNID controls, but their sVCAM-1 and sE-selectin titers did not differ from those of the other groups. There was no association between anti-streptococcal antibodies titers and adhesion molecules in the two groups of patients with TS. Interestingly, sVCAM-1 levels were significantly higher among ABGA-positive than among ABGA-negative adults with TS.

Adhesion molecules are expressed by endothelial cells and peripheral blood mononuclear cells. An increased titer of their soluble forms may either be related to an increased expression of their membrane-bound counterparts, or be due to enhanced proteolytic degradation from the cell surface following endothelial damage [20,27]. Overall, they are considered markers of a systemic inflammatory response, as well as of endothelial activation and damage.

Our findings support a concurrent inflammatory response in patients with TS of different age groups and duration of disease. These findings might be explained in different ways. First of all, it has been observed that anti-streptococcal antibody titers are significantly elevated in children and adults with TS [16–18], even though an ongoing GABHS infection or colonisation was never definitely confirmed by culture or polymerase chain reaction analyses from throat specimens or skin biopsies. Our findings could therefore be due to a bystander effect of an inflammatory microenvironment created by recurrent or persistent GABHS infection. Indeed, soluble adhesion molecules have been reported as persistently elevated in recurrent paediatric upper respiratory tract infections of viral origin [28], and the possibility of a subclinical GABHS pharyngeal carriage has been thoroughly documented [29]. However, if this was the case, one would expect adhesion molecule titers to correlate with ASOT or anti-DNAse B titers, both highly specific markers of an ongoing immune response to this agent. Consistent with previous reports, serum anti-streptococcal antibodies were raised in our TS patients, but did not covary with adhesion molecules, suggesting that the elevated levels of adhesion molecules may not be a mere epiphenomenon of a concomitant GABHS infection.

An alternative explanation for our findings is that TS is associated with an immune-mediated systemic inflammatory response. Adhesion molecules are increased in several immune-mediated diseases, such as rheumatoid arthritis [30] and systemic lupus erythematosus (SLE) [31], but also in inflammatory diseases primarily of the CNS, like MS or EAE [32–34]. In SLE, upregulation of adhesion molecules was linked to acute disease activity and chronic multi-organ damage, as well as to abnormal titers of antiphospholipid antibodies in SLE-related antiphospholipid syndrome [35]. Interestingly, serum sICAM-1 levels were reported as significantly elevated in children with RF, both during the acute and the remitting phases of the disease [21]. During remission, sICAM-1 titers remained high, while other acute phase reactants (erythrocyte sedimentation rate and fibrinogen) had already returned to normal [21]. Another group reported elevated levels of sICAM-1, sVCAM-1 and sE-selectin in the serum of patients with rheumatic mitral stenosis [36]. ABGA, a potential biological marker of post-streptococcal neuropsychiatric disease, are highly sensitive in discriminating children with SC, the neuropsychiatric manifestation of RF, from children with uncomplicated

streptococcal infections [26,37]. Moreover, ABGA were shown to be more frequent in a subgroup of patients with TS than in normal and pathological controls [18,38,39]. In our new cohort, we replicated the finding of a 25–30% proportion of ABGA positivity in patients with TS previously reported by our group [18]. Importantly, sVCAM-1 levels were significantly higher in ABGA-positive than in ABGA-negative adult patients with TS, although it was not possible to detect a quantitative correlation between the two markers, since Western immunoblotting, currently the most accurate technique in detecting ABGA [26], is not a quantitative assay. A possible conclusion is that adhesion molecules and ABGA are both markers of the same immune-mediated process, potentially triggered by a preceding GABHS infection, which might be relevant in a proportion of patients with TS. Consistent with this hypothesis, our SC/PANDAS cohort, similarly to the RF cohorts reported by other groups [21,36], showed significantly elevated levels of sICAM-1.

CNS inflammatory disorders are characterised by an increased expression of adhesion molecules on brain microvessel endothelial cells and glial cells within active lesions [20]. When expressed by endothelial cells in their membrane-bound form, these molecules act as ligands for leukocytes to facilitate their passage through the blood–brain barrier (BBB). Soluble forms of adhesion molecules are detectable in the serum and CSF of MS patients, and seem to correlate with the degree of activity of the inflammatory response in the CNS [32–34]. ABGA have recently been documented in the CSF of patients with SC [40] or a post-streptococcal encephalitis lethargica-like syndrome [41], suggesting the possibility of intrathecal synthesis of these antibodies. To the best of our knowledge, in serum ABGA-positive patients with TS, a condition in which the BBB is known to be intact, a similar finding has not been confirmed, due to the poor availability of CSF specimens from these patients. The increased levels of soluble adhesion molecules found in our patients might be related to brain microvessel endothelial activation, thereby facilitating lymphocytic extravasation in the brain, even in the absence of a major disruption of the BBB. Analysis of soluble adhesion molecules and ABGA in the CSF of patients with TS is warranted to verify this hypothesis.

The lack of correlation between adhesion molecules titers and disease severity in TS patients does not exclude the contribution of an underlying immune-mediated process to the pathophysiology of the disease. In fact, a similar mismatch was already reported in patients with RF [21] or in a group of 57 patients with neuropsychiatric SLE [35]. In addition, it has to be pointed out that severity scales for neurobehavioural disorders, such as TS, provide ordinal data which are only approximately continuous, and therefore not ideal for correlation analyses with biological measures. However, even after categorization of YTSS scores, the most used and reproducible severity scale for tics in TS, we failed to detect a significant association with our biological markers. A single-point-in-time study design, as is ours, does not detect possible covariations over time of clinical features and biological measures. A longitudinal type of study would be necessary for this purpose, and it is currently being performed by our group.

The observed differential involvement of adhesion molecules between TS and SC/PANDAS might result from differences in the immune-mediated processes underpinning the two conditions. There is evidence for a variable regulation in the expression of these molecules, which might account for different serum levels according to the temporal stage of the inflammatory process [42,43]. ICAM-1 and E-selectin are mainly stimulated by T-helper-1 lymphocyte-secreted cytokines (IL-1, TNFα, IFN-γ) [42], whereas antiinflammatory cytokines, such as IL-4, IL-10 and IL-13, secreted by T-helper-2 lymphocytes, have been shown to downregulate ICAM-1 and E-selectin synthesis and facilitate VCAM-1 expression [44]. Our SC/PANDAS patients were all characterised by a recent acute onset of neuropsychiatric symptoms, suggesting that blood samples were collected during the acute phase of inflammation; conversely, all TS patients had a chronic illness. In the present study, we did not perform cytokine measurements; therefore, additional comments on the different pattern of expression of adhesion molecules in TS and SC/PANDAS are merely speculative. The question of a differential cytokine expression during the course of TS should be addressed in future studies.

In conclusion, our study gives preliminary evidence for the involvement of adhesion molecules in TS, thereby suggesting the presence of a chronic systemic immune-mediated response in this condition. Further work is warranted to investigate the contribution of other systemic immunological markers in TS, as well as the involvement of the intrathecal district in such immune response. Moreover, longitudinal surveys are currently ongoing, in order to evaluate the extent of temporal covariation of clinical features and immunological measures in patients with TS.

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Increased Antistreptococcal Antibody Titers and Anti–Basal Ganglia Antibodies in Patients With Tourette Syndrome: Controlled Cross-Sectional Study

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ABSTRACT

The association between Tourette syndrome, attention-deficit hyperactivity disorder (ADHD), and obsessive-compulsive disorder following streptococcal infections has been documented, but with conflicting reports. We thus felt it was important to investigate this association in a group of Italian patients not previously documented. We took blood on 69 patients with Tourette syndrome and 72 age- and sex-matched tic-free controls. Laboratory staff were blind to the diagnostic status of the subjects. Evidence of recent streptococcal infection was defined using antistreptolysin titers. Anti–basal ganglia antibodies were determined using human basal ganglia sections. Statistical analysis was conducted using analysis of variance and chi-square tests. Raised antistreptolysin titers were found in 41 of 69 (59%) patients with Tourette syndrome and 14 of 72 (19%) controls ($P = .000$). Positive anti–basal ganglia antibodies were found in 22 of 69 (32%) subjects with Tourette syndrome compared with 7 of 72 (10%) controls, which was also significant ($P = .002$). Raised antistreptolysin titers were detected in 18 of 22 (82%) patients with Tourette syndrome with positive anti–basal ganglia antibodies and 22 of 47 (47%) patients with negative anti–basal ganglia antibodies ($P = .01$). These results support the reported association between streptococcal infection and anti–basal ganglia antibodies and some patients with Tourette syndrome. (*J Child Neurol* 2006;21:000–000; DOI 10.2310/7010.2006.00178).

Gilles de la Tourette syndrome or Tourette syndrome is characterized by the presence of chronic multiple motor abnormalities and one or more vocal tics, which must be present for at least a year[1,2] and characteristically wax and wane over time. Comorbid neuropsychiatric symptoms include obsessive-compulsive behaviors and disorder and attention-deficit hyperactivity disorder (ADHD).[3,4]

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Epidemiologic studies have shown that Tourette syndrome is relatively common, occurring in between 0.4% and 1.76% of children between the ages of 5 and 18 years.[5–11] The prevalence is even higher in individuals with autistic spectrum disorder[12] and individuals with learning disability and/or mental retardation.[13]

The etiology of Tourette syndrome remains unclear, although a combination of genetic and environmental (nongenetic) factors has been suggested.[14–17] Not only was Tourette syndrome found to be genetic, but autosomal dominant with a single major gene with incomplete penetrance was documented. However, much of the genome was then excluded, and subsequent genome scans and investigations have suggested regions of interest on chromosomes 2, 4, 5, 7, 8, 10, 11, 13, and 17, as well as the *DRD4* and *MAOA* genes.[18,19] For thorough reviews on the genetics of Tourette syndrome, the reader is referred to Pauls.[14,15] It has also been proposed that although Tourette syndrome is an inherited disorder, environmental factors either have an etiologic role or mediate the severity of the phenotype.[20] Therefore, other etiologic theories were proposed and investigated.

It was suggested that group A β-hemolytic streptococcal infections might be important factors in acute-onset neuropsychiatric and movement disorders.[21] Sydenham chorea is considered the prototype of an autoimmune disorder triggered by an infectious agent. Thus, Sydenham chorea is documented as having a clearly defined association with rheumatic fever with a preceding group A β-hemolytic streptococcal infection.[22,23] In addition to the chorea that typically involves the face and extremities, affected individuals can also present with behavioral or emotional difficulties, which can predate the motor abnormalities by weeks to months.[24,25] Then in 1998, Swedo et al proposed that Sydenham chorea was not the only immune-mediated central nervous system manifestation of group A beta-hemolytic streptococcal infections and proposed diagnostic criteria for a group of patients with the acronym PANDAS (pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections).[21]

The proposed mechanism in Sydenham chorea and PANDAS is the presence of cross-reactive antibodies induced by group A β-hemolytic streptococcal infections that bind specifically to basal ganglia antigens.[26] The presence of anti–basal ganglia antibodies in patients with Sydenham chorea[27] supports this hypothesis.

In a study of nine patients with Sydenham chorea (acute and matched convalescent individuals), monoclonal antibodies showed specificity for mammalian lysoganglioside and *N*-acetyl-beta-D-glucosamine, the dominant epitope of the group A streptococcal carbohydrate.[28] Sydenham chorea antibodies targeted the surface of human neuronal cells, with specific induction of calcium/calmodulin-dependent protein kinase II activity by monoclonal antibody 24.3.1 and sera from patients with active chorea. Convalescent sera, as well as sera from other streptococcal diseases in the absence of chorea (acute rheumatic fever), did not activate the kinase. This evidence implicates antibody-mediated neuronal cell signaling in the immunopathogenesis of Sydenham chorea, which might improve our understanding of antibody-mediated neurologic disorders.[28]

We decided to investigate the hypothesis that Tourette syndrome might be associated with group A β-hemolytic streptococcal infections and anti–basal ganglia antibodies in a cohort of children and adolescents with Tourette syndrome and compare them with a group of healthy control children from similar sociodemographic backgrounds. Neither group had been previously documented.

MATERIALS AND METHODS

Ethical permission for the study was given by the University of Catania Hospital Ethics Committee. The parents of each child gave written informed consent. Sixty-nine consecutive outpatients with Tourette syndrome attending a referral-based clinic in our department (64 boys, 5 girls; age range 7–15 years) participated in the study. Seventy-two healthy control subjects also participated. The patients were interviewed by one of us (R.R.), who used the following standardized instruments after training with another (M.M.R.): The National Hospital Interview Schedule[29] and the Yale Global Tic Severity Scale.[30] All patients fulfilled the diagnostic criteria for Tourette syndrome (defined by the Tourette Syndrome Classification Study Group)[31] and the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (*DSM-IV-TR*).[1] Comorbid diagnosis of obsessive-compulsive disorder and ADHD was made according to the *DSM-IV-TR* criteria. Obsessive-compulsive disorder was also diagnosed using the National Hospital Interview Schedule,[29] as well as the child version of the Yale-Brown Obsessive Compulsive Scale.[32]

Subject Selection

No subject had a history of rheumatic fever, Sydenham chorea, or known autoimmune disease. All patients with Tourette syndrome had normal neurologic examinations, normal cognitive functioning, and no evidence of psychosis. Patients were not selected as to whether they had had a clinical course consistent with infection-triggered exacerbations. Patients had not suffered an upper respiratory infection for at least a month prior to the study. Patients were medication free for at least 2 weeks prior to the study, which was undertaken in autumn and winter.

The tic-free control group consisted of 72 children (65 boys, 7 girls; age range 7–15 years) recruited from the same hospital clinics (with similar sociodemographic characteristics), including 34 neurologic patients (n = 19 patients with epilepsy; n = 15 patients with mental retardation [learning disability]) and 38 normal healthy controls, that is, youngsters presenting for minor ailments to the walk-in clinic who received no relevant or major medical or neuropsychiatric diagnosis, such as psychosis. No throat or streptococcal infections were documented in the control subjects for at least 1 month before the study.

Laboratory Investigations

Blood was obtained from subjects and controls and was analyzed by personnel blind to their diagnostic status. Statistical comparisons were not performed until all of the investigations had been completed. Owing to the high incidence of both false-positive throat cultures in subjects who are not acutely infected and false-negative findings in subjects acutely infected with streptococcus,[33] throat cultures were not obtained.

Recent group A β-hemolytic streptococcal infections were defined using antistreptolysin O titers. Indirect immunofluorescence[34] revealed antibody binding to anti–basal ganglia antibodies. Anti–basal ganglia antibodies were determined using human basal ganglia sections according to Husby et al and scored using the criteria described therein.[27] An antistreptolysin titer cutoff limit of > 400 IU/L was used in our study.[35]

Statistical Analysis

Statistical analysis was performed as follows: sex distribution was analyzed using the chi-square test and analysis of variance to compare the age differences between the clinical group and the control group. To compare the antistreptolysin titers between patients with Tourette syndrome and the healthy control group, different distributions of increased values between the groups were analyzed using chi-square, as well as for comparisons between patients with Tourette syndrome and the control group positive for anti–basal ganglia antibodies. The *PRIMER Statistical Package for Biomedical Sciences* was employed.[36]

RESULTS

There were 69 patients with Tourette syndrome (64 male) and 72 healthy controls (65 male) (not significantly different; P = .822). The mean age of the patients was 9.36 years (SD 3.18 years) and that of the normal controls was 8.75 years (SD 2.75 years) (not significantly different; P = .225). The mean age at onset of tics was 6.6 years (range 2–15 years), and 65% had a positive family history of tics. The mean severity score of the tic symptoms,

measured using the Yale Global Tic Severity Scale, was 26.3% (range 0–79%). Eleven patients reported that their symptoms were worse with fever; the symptoms of 27 patients (37%) improved during summer months.

In 23 of 69 (33%) children with Tourette syndrome, a comorbid diagnosis of ADHD was made. Obsessive-compulsive disorder was diagnosed in 10 of 69 (14%) patients with Tourette syndrome.

Raised antistreptolysin titers (> 400 IU/mL) were found in 41 of 69 (59%) children with Tourette syndrome but in only 14 of 72 (19%) subjects in the control group (significant; $P = .000$). Positive anti–basal ganglia antibodies were found in 22 of 69 (32%) children with Tourette syndrome compared with 7 of 72 (10%) subjects in the control group (significant; $P = .002$). Raised antistreptolysin titers were detected in 18 of 22 (82%) patients with positive anti–basal ganglia antibodies and 22 of 47 (47%) of those with negative anti–basal ganglia antibodies (significant; $P = .01$).

DISCUSSION

The mean age at onset of symptoms in our patients was 6.6 years, and there was a male predominance, consistent with published data.[3,20]

As mentioned, the etiology of Tourette syndrome is complex, with genetics and pre- and perinatal theories invoked but no conclusive evidence being documented. New evidence was thus sought, and the role of infections and neuroimmunologic factors in the etiopathogenesis of Tourette syndrome was proposed.[21,37]

Swedo and colleagues were the first to describe PANDAS in a group of 50 patients.[21] They proposed specific diagnostic criteria: (1) the presence of obsessive-compulsive disorder and/or tic disorder; (2) pediatric onset (symptoms evident between 3 years and puberty; (3) episodic course of symptom severity and clinical course characterized by an abrupt onset or dramatic exacerbation of symptoms; (4) symptoms bearing a temporal relationship to group A β-hemolytic streptococcal infection; and (5) an association of neurologic abnormalities, for example, motor hyperactivity and "adventitious" movements. Kurlan discussed the suggestion that tic disorders and associated behavioral disturbances (eg, obsessive-compulsive disorder) might develop following streptococcal infections by the process of molecular mimicry, whereby antibodies are directed against bacterial antigens cross-reacting with brain targets.[38]

Dale et al proposed that neuronal membrane glycolytic enzymes might be putative autoantigens in post-streptococcal neuropsychiatric disease, demonstrating that autoantibodies can affect neuronal metabolism.[39] They noted that these enzymes exist on streptococcal surfaces, sharing about 25% to 50% homology with human glycolytic enzymes, therefore representing a theoretic model of molecular mimicry.[39]

Our results give further support to a role for group A β-hemolytic streptococcal infections in a subgroup of patients with Tourette syndrome. The diagnoses of Tourette syndrome were made by an expert, using standardized assessment procedures and internationally accepted diagnostic criteria.[1]

However, it is important to note that the validity of PANDAS and the relationships between tic disorders, Tourette syndrome, and/or obsessive-compulsive disorders and group A β-hemolytic streptococcal infections have become controversial. Thus, some scientific groups have reported results broadly in favor of the neuroimmunologic hypothesis, showing that in some patients with Tourette syndrome, there is evidence of group A β-hemolytic streptococcal infections and/or some patients with Tourette syndrome have increased anti–basal ganglia antibodies in case reports,[40] cohort studies,[41] and controlled studies.[33,34,41–45]

Peterson et al documented antistreptolysin titers and basal ganglia volumes in 105 individuals (7–55 years) with *DSM-IV* chronic tic disorder and obsessive-compulsive disorder and compared them with 37 age-, sex- and socioeconomic status–matched controls.[46] A *DSM-IV* diagnosis of ADHD was associated significantly with titers of two distinct antistreptococcal antibodies: antistreptolysin O and antideoxyribonuclease B, which was significant after controlling for the effects of chronic tic disorder and obsessive-compulsive disorder. No significant association was seen between antibody titers and a diagnosis of chronic tic disorder or obsessive-compulsive disorder. They suggested that previous reports of an association between antistreptococcal antibodies and chronic tic disorder and obsessive-compulsive disorder might have been confounded by the presence of ADHD.

Thus, in studies to date, 522 patients previously reported (Tourette syndrome = 242; tics = 150; tics or obsessive compulsive disorder = 25; tics, obsessive-compulsive disorder, and ADHD = 105) and our 69 patients with Tourette syndrome (591 patients from six independent groups in eight studies) have all shown evidence of streptococcal infections in patients with Tourette syndrome, tics, obsessive-compulsive disorder, and ADHD (Table 2). It is acknowledged that the cutoff points used in studies have varied[41,43,44,46]; nevertheless, the results obtained are reasonably consistent. 5

In our study, 23 of 69 (33%) subjects had ADHD and 10 of 69 (14%) subjects had obsessive-compulsive disorder. As these numbers were too small for meaningful analysis of subgroups within the patients, we undertook no further analysis. Whereas some studies reported fewer streptococcal infections in patients with tics and/or Tourette syndrome and obsessive-compulsive disorder compared with those with ADHD,[41,46] others found no relationship between streptococcal infections and these comorbidities.[33,34,45] Other centers failed to compare the Tourette syndrome–alone group with those with comorbid disorders.[43,44]

The simultaneous obtaining of throat cultures is possibly desirable, although some studies suggest that this is not so[33];

Table 1. Comparison Between Anti–Basal Ganglia Antibody–Positive and Anti–Basal Ganglia Antibody–Negative Patients With Tourette Syndrome and the Control Group 8

	Antineural Antibody Positivity	*Absence of Antineural Antibodies*	*Total*
Tourette syndrome patients, *n* (%)	22 (31.88)	47	69 (100)
Control group, *n* (%)	7 (9.72)	65	72 (100)
P value	.002		151 9

Table 2. Studies Reporting Neuroimmunologic Influences in Neuropsychiatric Disorders, Excluding PANDAS

Study	Year	Number of Patients	Patients' Diagnoses
Peterson et al[46]	2000	105	Tic, obsessive-compulsive disorder, attention-deficit hyperactivity disorder (ADHD)
Muller et al[43]	2000	36	Tourette syndrome
Muller et al[33]	2001	25	Tourette syndrome
Cardona and Orefici[44]	2001	150	Tics
Morshed et al[45]	2001	80	Tourette syndrome
Church et al[34]	2003	100	Tourette syndrome
Murphy et al[41]	2004	25	Tics, obsessive-compulsive disorder
Rizzo et al (present study)	2004	69	Tourette syndrome
Total		591	

PANDAS = pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections.

therefore, we did not obtain them. This was also not undertaken in several investigations.[34,46]

There are, however, conflicting results as to the association between group A β-hemolytic streptococcal infections and Tourette syndrome. Data from two other groups including reviewing medical records[47] and controlled studies[48,49] failed to show any significant relationship between group A β-hemolytic streptococcal infections and/or the presence of anti–basal ganglia antibodies in patients with Tourette syndrome. As in two of the studies, the numbers are identical and originate from the same laboratory; it is unclear as to whether the 41 children in the two studies are the same or independent cohorts. More recently, the same group failed to demonstrate anti–basal ganglia antibodies in 15 children with PANDAS when comparing them with 15 controls.[50] Furthermore, the opinions of others in editorials and/or commentaries do not support the relationship between group A β-hemolytic streptococcal infections and/or neuronal anti–basal ganglia antibodies or the PANDAS/autoimmune hypothesis.[51,52]

Moreover, even within laboratories, somewhat differing results have been documented. Thus, Singer et al studied 41 children with Tourette syndrome and 39 controls and reported that the patients had significantly higher serum levels of antineuronal antibodies against the putamen but not the caudate or globus pallidus; nevertheless, they concluded that their results were not in support of the hypothesis and suggested that a relationship between the antineuronal antibodies, clinical characteristics, and markers for streptococcal infections remained equivocal.[53] Further, Singer et al reported that 11% of children with tic disorders described abrupt changes in tic behavior soon after streptococcal infections.[54] Similarly, Wendlandt et al studied antineuronal antibodies from 20 children with Tourette syndrome and 21 controls using Western blot techniques.[55] There were significant differences between patients' and controls' blots, and these were identified using striatal epitopes. This was in contrast to similar patterns found in the groups for the globus pallidus, muscle, and HTB-10 tissue. Thus, these results appear, in our opinion, to support striatal autoimmune involvement in Tourette syndrome pathophysiology[55] in some patients and thus are broadly in agreement with the hypothesis suggesting a relationship between group A β-hemolytic streptococcal infections and an autoimmune factor, although, as mentioned, three earlier documentations from the Singer et al group are not in favor of and/or do not support the hypothesis. Thus, the five studies that do not support the role of group A β-hemolytic streptococcal infections in various relevant patients include four from Singer et al's group (three cohorts of 41 patients and the Wendlandt et al study including 20 patients[55]) and one other study (Betancourt et al[47]), documenting 38 patients, but some of these appear to be inconsistent. 6

What are the possible reasons for the differing results? These might be explained by at least four differences in laboratory techniques.[56] Pavone et al documented significant differences in antibrain antibodies in 22 patients who met the strict criteria for PANDAS (mean age 10.1 years) when compared with 22 patients (mean age 9.1 years) with clinical evidence of active group A β-hemolytic streptococcal infection, confirmed by throat culture and elevated antistreptolysin titers, but without a history or clinical evidence of tics or obsessive-compulsive disorder.[57] These results suggest that antibrain antibodies that are present in children with PANDAS cannot be explained merely by a history of group A β-hemolytic streptococcal infection.

Further, most studies have not commented on the prevalence of group A streptococcal carrier status in the communities or countries where the studies were undertaken[34,43–45] or taken into account geographic or seasonal variations. For example, in the Morshed et al study, patients with Tourette syndrome were recruited from the United States, SC patients from Brazil, those with autoimmune disorders from Japan, and controls from the United States.[45] Some studies have included season as a covariate and found no significant effect,[46] whereas others did not mention the season of study.[34,42–44] 7

In this context, studies on group A streptococcus could shed light on these difficulties. In an urban longitudinal study of between 48 and 100 children, 5658 throat cultures were performed and 878 (15.5%) were positive for group A streptococcus.[58] Kaplan et al documented that between 29% and 45% of 500 children in a semiclosed community had group A streptococcus yielded on throat cultures; seasonal variations were reported.[59] In another study, both geographic and seasonal variations in group A streptococcus were demonstrated.[60] In Italy, in 1982, D'Arca et al investigated 1535 primary school children in Rome over 2 years, demonstrating that group A streptococcal carrier status was between 11.7% and 19.6%.[61] A recent study in Italy documented that 620 of 321,612 hospitalized children (0.19%) had severe streptococcal diseases.[62] It is acknowledged that the former figures are old and the latter are of hospitalized children. Nevertheless, they give some indication that the true prevalence of group A streptococcal carrier states and infections was not known in Italy at the time of our study. No other PANDAS studies to date have given prevalence rates of

group A streptococcal carrier states in the geographic area of the study at the time of the investigations, which might have a bearing on conflicting results.

Thus, the PANDAS hypothesis or the role of the group A β-hemolytic streptococcal hypothesis in patients with Tourette syndrome and tics is far from simple, and further controlled and longitudinal studies with larger numbers of patients supporting or refuting the hypothesis are important to examine more precisely the role of group A βhemolytic streptococcal infections in neuropsychiatric disorders. Studies as to the precise epidemiology of group A streptococcal carrier status would also be helpful in understanding the hypothesis and published results.

Possible limitations to our study include the following: (1) our nonselection of patients who had a Tourette syndrome course consistent with infection-triggered exacerbations, (2) blood was taken without relevance to onset or exacerbation of neuropsychiatric symptoms, (3) we employed no throat cultures, and (4) we did not analyze the different diagnostic groups (ADHD, obsessive-compulsive disorder) because we felt that with the small numbers (obsessive-compulsive disorder = 10), it would be inappropriate. Nevertheless, our data add to the further understanding of the relationship between group A β-hemolytic streptococcal infections and Tourette syndrome.

CONCLUSIONS

In conclusion, our results on 69 patients with Tourette syndrome are in broad agreement with the majority of laboratories (using 522 patients), suggesting a relationship between a group A β-hemolytic streptococcal infection and some patients with Tourette syndrome. We have also documented that some patients with Tourette syndrome have anti–basal ganglia antibodies. We do not suggest that group A β-hemolytic streptococcal infections cause Tourette syndrome. It is conceivable that an individual inherits susceptibility to Tourette syndrome and also to the way that the individual reacts to group A β-hemolytic streptococcal infections. To our knowledge, many genetic studies, as well as prospective studies evaluating the role of environmental factors (pre- and perinatal) in the etiopathology of Tourette syndrome, are under way to explore further the etiopathogenesis of Tourette syndrome. Only when the results of these studies, as well as further studies investigating the role of streptococcal infections and indeed PANDAS, are available will the actual role of group A β-hemolytic streptococcal infections in the etiopathology of Tourette syndrome be clarified further.

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NEUROIMAGING IN

GILLES DE LA TOURETTE SYNDROME

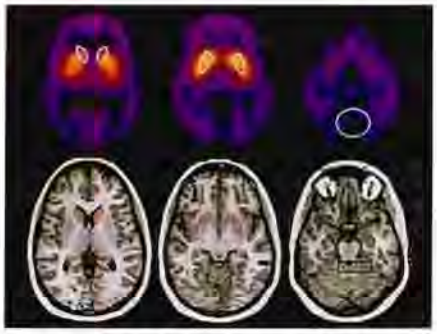
D2 be or not to be?

Neuroimaging of monozygotic twins with Tourette syndrome suggests that increased binding by dopamine D2 receptors contributes to the disease phenotype.

MARY M. ROBERTSON

Tourette syndrome (TS), a chronic tic disorder, has been known for more than 150 years. Although it remains underrecognized, literature on TS has mushroomed as scientists continue to explore the plethora of symptoms and their underlying pathophysiology. Mainly on the basis of empirical evidence, it has been postulated that altered dopamine function contributes to the Tourette phenotype. Dopamine antagonists have beneficial effects whereas dopamimetic agents exacerbate symptoms; dopamine metabolites in cerebrospinal fluid are decreased in some TS patients[1]. However, there is little hard evidence supporting the involvement of dopamine and functional neuroimaging (FNI) studies have so far been unable to resolve the debate. In a recent issue of *Science*, Wolf and colleagues[2] have made a timely contribution to understanding the etiology of TS by combining genetic, neuroimaging and neurobiochemical approaches to the study of five sets of monozygotic (MZ) twins, concordant for TS but discordant for symptom severity.

TS, characterized by multiple motor and one or more vocal tics, is genetically determined, although the precise inheritance pattern is unknown. Studies have suggested autosomal dominance, but more than 80 percent of the genome (including loci for dopamine D1 and D2 receptors) has so far been excluded[3–5]. Prenatal environmental factors such as lower birth weight have also been invoked as determinants of phenotype[6]. TS prevalence is 0.5 per 1000 live births, and it occurs three to four times more often in males than females. Symptoms, including coprophenomena (inappropriate involuntary swearing), echophenomena (copying behaviors) and palilalia (repeating oneself), are heterogeneous. Many tics are clearly involuntary, and TS patients with mild disorder are often unaware that they are "ticcing." Some patients with premonitory sensations, "have to" perform tics to relieve local physical tensions (previously likened to a sneeze). Yet other TS subjects perform tics such as forced touching in a compulsive way, and with this group, the boundary with compulsive behavior is indistinct. It is generally recognized that chronic multiple tic disorder and obsessive-compulsive behaviors are part of TS and probably both phenotypes of the putative TS gene(s). A disease as curious as "full-blown" TS will always find important individuals with the disorder to give it credibility and raise awareness of it. In this context, Samuel Johnson (correctly) and Mozart (in my opinion, incorrectly) have both been suggested to have suffered from TS.



Time-averaged SPECT images of IBZM binding in a single Tourette patient (*top row*). Regions of interest (red) created on the MRI scans (*bottom row*) for the caudate nucleus (left), putamen (middle) and cerebellum (right), are shown transposed (white) onto the SPECT images. (Reprinted with permission from ref. 2. ©1996 American Association for the Advancement of Science.)

In their study, Wolf and co-workers used single-photon emission computed tomography (SPECT) combined with magnetic resonance imaging (MRI) to measure binding of the radioligand iodobenzamide (IBZM), a potent dopamine D2 receptor antagonist, in the striatal dopaminergic pathway. Their technique measures, *inter alia*, the availability of D2 receptors in different regions of the striatum. IBZM binding in the caudate nucleus (CN) was significantly greater (17%) in all five of the more affected twins. MZ twin intrapair differences in D2 receptor binding in the CN correlated significantly with clinical severity ratings; in contrast, no significant differences in binding were found for the putamen (the region adjacent to the CN). The authors also analyzed all subjects with brain perfusion SPECT and found no significant intrapair differences, demonstrating that their findings were not attributable to regional cerebral blood flow effects. They postulate that their results support the notion that D2 receptor "supersensitivity" explains the phenotypic variations observed in TS patients. Although the basis for increased D2 receptor binding is unclear, they speculate that it might reflect "increased receptor density, receptor-ligand affinity, endogenous dopamine concentrations, or any combination of these."

This group is not the first to study the D2 receptor or the striatal pathway in TS subjects; many others have done so, but with conflicting results. Although studies have demonstrated hypoperfusion of the left CN and cingulate, as well as the left medial temporal region, to be related to tic severity, perfusion patterns were not characteristic of any behavioral subgroups[7]. Some[8,9] but not others[10] have found increases in presynaptic dopamine transporters in TS patients. The same inconsistency is apparent at the level of the D2 receptor, with some groups[10,11] showing no difference from controls and the Wolf study[2] demonstrating intrapair differences among MZ twins. The latter finding seems to complement postmortem studies showing significantly greater radioligand binding by striatal presynaptic D2 receptors in three TS cases[12]; neuronal dopamine uptake carrier sites were significantly increased in number over control values by 37 percent in the CN and 50 percent in the putamen. Levels of dopamine and its primary metabolites were normal and only slight alterations in D1 and D2 receptor binding were observed[12], probably due to medication.

Many clinical scientists view FNI as a computing "black box," the science of which may be hard to validate. In this context, there are problems with the Wolf *et al.* study. The TS patients were heterogeneous regarding medication; ideally, the patients should have been neuroleptic-naive. As it is, five were neuroleptic-naive, three were drug-free for three or more years, and two were receiving neuroleptics just six weeks be-

fore the study. Effects of acute neuroleptic treatment on the dopaminergic presynaptic projections are well established, but the effects of chronic treatment are uncertain[10]. Because individual CN binding values varied substantially between more and less affected pairs, it would have been informative to know the relation, if any, between binding values and medication status. If medication status could affect the D2 receptor binding data, then the entire result might be explained on the basis of the medication history rather than symptom severity. In addition, IBZM binding is age-related in normal controls[13], yet only two pairs were matched for age (31 years); the other three pairs were in three different decades. However, the intrapair D2 receptor binding differences and their correlation to symptom severity answer this question to some extent. Surprisingly, no mention is made in the article of the volumes of the CN or the putamen. Several studies using volumetric MRI show abnormalities in CN size among Tourette patients[14] and also between MZ twins with TS (ref. 15). Neither is reference made to possible differences between right and left hemispheres. These last two concerns are all the more surprising as the same group has previously shown that, in a similar cohort of twins (concordant for TS, discordant for severity), right CN volume was slightly but significantly reduced in more severely affected twins[15]. Inclusion of age- and sex-matched healthy controls in the Wolf study would have helped to put previous conflicting results into context, which is always important given the small numbers of patients studied and the clinical heterogeneity of the condition.

What then distinguishes the investigation by Wolf and colleagues? They must be congratulated on their design, using MZ twins (who share identical genetic material and an almost identical pre- and perinatal environment) and using three independent neuroimaging techniques, making this twin cohort study unique. It is also noteworthy that the male:female ratio in the study is similar to that encountered in the general population. Future investigations should also include FNI analyses of unaffected twins together with normal controls. If the findings of Wolf *et al.* can be replicated, then they will have important implications beyond TS. This study[2] and that of Hyde *et al.*[15] (although possibly using some of the same twin pairs) highlight the brain's structural and functional plasticity in response to environmental influences, which results in different clinical presentations (phenotypes).

The findings of the Wolf study pose many questions. If biological differences accompany variation in symptom severity, then is there any hope of accurately studying other phenotypes when many arise at different times in the course of the disorder and some may be more difficult to measure than severity? Are the observed differences in D2 receptor binding a result of a bystander phenomenon or or do they tell us something about the primary lesion? How will the Wolf data compare with previous conflicting norm-referenced studies[7,11]? In addition, one should not forget the role of other neurotransmitters in the dopaminergic striatal circuits implicated in TS — what role might, for example, serotonin or γ-aminobutyric acid play?

Kinnear Wilson dubbed the components of the basal ganglia (a region that includes the CN and putamen) "those dark basements of the brain." FNI appears to be casting light on the physiology and pathology of certain of these dark basements. If indeed the components of the basal ganglia do constitute "the orchestra of movement disorders," is CN the conductor of Gilles de la Tourette's neuropsychiatric symphony?

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Estrogen and bone: New pieces to the puzzle

Recent studies on osteoclast apoptosis and TGF-β gene regulation provide new insights into the mechanisms by which estrogens and their analogues inhibit bone resorption (pages 1132–1136).

LAWRENCE G. RAISZ

Although it has long been known that estrogen withdrawal stimulates osteoclasts to resorb bone resulting in bone loss and that estrogen replacement halts bone resorption, the mechanisms mediating these effects remain an enigma. It has been difficult to demonstrate inhibition of bone resorption in bone organ culture systems or in isolated osteoclast preparations[1,2], which suggests that estrogens act indirectly either on osteoclast precursors or on cells of the osteoblast lineage that are necessary for osteoclast activation. Estrogen may exert its indirect effects by altering the production of local regulatory cytokines possibly in the bone marrow where osteoclast and osteoblast precursors reside. Factors se-

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BRAIN PERFUSION PATTERNS WITH Tc-99m-HMPAO/SPET IN PATIENTS WITH GILLES de la TOURETTE SYNDROME.

Gilles de la Tourette Syndrome (GTS) has an estimated prevalence of 0.5 per thousand giving figures of 110,000 patients in the USA and 27,500 in the UK. The biochemical aetiology is unknown but it has been suggested that the basal ganglia are implicated in the pathophysiology of GTS. CT scanning shows no consistent structural abnormality.

We studied 25 GTS patients with high resolution gamma camera HMPAO/SPET, paying particular attention to the perfusion of the basal ganglia. All patients had a wide range of motor and vocal "tics", and ages from 7 to 48 years. 6 were female. A bank of 10 normal HMPAO/SPET studies were used as control.

HMPAO/SPET was carried out with the IGE 400AC/STARCAM system using routine protocols for acquisition, reconstruction and analysis of the data. 10 MBq/kg (patient weight) of HMPAO was injected (i.v.) after a full explanation of the procedure and written consent signed. This study was approved by the local ethical committee.

Qualitative analyisis of transverse and coronal slices revealed, for all patients, a significant decrease in the tracer concentration in the basal ganglia nd thalami. There were perfusion deficits identifiable in the cortex of the frontal and particularly the temporal lobes. Semi-quantitative analysis with normalisation for the cerebellum confirmed these findings.

Conclusion: for the first time perfusion deficits are shown with high resolution gamma camera HMPAO/SPET in the basal gaglia of patients with GTS, which may help to explain the extrapyramidal motor and vocal abnormalities found in these patients.

.. HALL M, COSTA DC, SHIELDS J, HEAVENS J, ROBERTSON M, ELL P (1990) Brain perfusion p... with 99 Tc HMPAO/SPET in patients with Gilles de la Tourette syndrome Nuclear Medicine Suppl 27, 243-245

"BRAIN PERFUSION PATTERNS WITH $^{99}Tc^{m}$-HMPAO/SPET IN PATIENTS WITH GILLES de la TOURETTE SYNDROME - SHORT REPORT"

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Introduction - In 1885 George Albert Brutus Gilles de la Tourette described a syndrome consisting of involuntary vocalisations, namely coprolalia, echolalia, echopraxia and a variety of complicated movements. These clinical characteristics seem to be independent of culture, as they occur with some uniformity, irrespective of the country of origin. The age of onset ranges from 2 to 15 years, with no sex predominance.

The prevalence of Gilles de la Tourette syndrome (GTS) is 0.5 per thousand of population with an estimated total number of 110,000 patients in the U.S.A. and approximately 27,500 in the U.K.

Genetic inheritance has been reported by Robertson, 1989. However, the etiology is unknown and there has been no consistent pathologic findings to explain symptoms and signs characteristic of this syndrome. Although basal ganglia and brain cortex pathology has been suggested, CT scan showed no consistent structural abnormalities, Robertson, 1989.

This study was designed to investigate the perfusion patterns of the brain in patients with GTS, paying particular attention to the brain cortex and deep grey matter nuclei (basal ganglia and thalamus).

Patients - 25 GTS patients with an age range from 7 to 48 years were studied. 19 were male and 6 female. All patients presented with a wide range of motor and vocal tics characteristic of the disease. 60% of the patients had a family history of tics, and 40% family history of obsessive compulsive disorder (OCD). OCD was present in 60% of the GTS patients, whilst attention deficit disorder (ADD) was present in 50% of them. Echophenomena (echolalia and echopraxia) was found in 56% of the total number of patients. 48% had coprolalia and copropraxia.

A small percentage (< 10%) of GTS patients were on treatment at time of SPET studies. All of these had obsessive compulsive disorder and 2 of them attention deficit disorder with hyperactivity.

Materials and Methods - 10 MBq per kg of patient weight (average adult dose = 740 MBq) was injected intravenously within 10 minutes of preparation. Patients were usually sitting in a comfortable position with the eyes open in a normal light room during the injection. For the majority of the cases (more than 90%) there were several involuntary movements before, during and immediately after the injection, that were reduced by the time patients were lying on the gamma camera couch. Single photon emission tomography (SPET) was started 15 minutes p.i. or longer, according to the time taken by each GTS patient to feel comfortable and relatively still.

The IGE 400 AC/STARCAM was used to carry out SPET following routine acquisition and processing protocols as previously described by our group, Costa et al., 1988. Three patients with very severe and frequent involuntary head movements underwent short time acquisition (5 and/or 10 minutes total acquisition) SPET performed with the IGE NEUROCAM, a three detector gamma camera under evaluation at our Institute, Townsend, 1990. One young (12 year old male) patient was studied with the IGE 400 AC/STARCAM and the SME 810.

Results - Qualitative analysis of transverse (parallel to the orbito meatal - OM - line), coronal and sagittal sections of the brain demonstrated a wide range of perfusion deficits in the cortex of frontal, parietal and temporal lobes. The visual cortex always showed a normal perfusion pattern. There were no changes in the tracer distribution in the cerebellar hemispheres. The cortical perfusion deficits were not suggestive of cerebral vascular disease, as they were not wedge shaped. Lateral ventricules were seldomly enlarged.

Patients with more severe disease (intensity and frequency of involuntary movements) showed marked reduction in the perfusion to the frontal and posterior parietal lobes (fig. 1), irrespective of the type of tics.

There were, however, consistent abnormalities found in the mesial cortex of the temporal lobes (perfusion deficits), as well as in the basal ganglia, particularly the heads of the caudate nuclei. These abnormalities were observed as either symmetric or more frequently asymmetric deficits in perfusion.

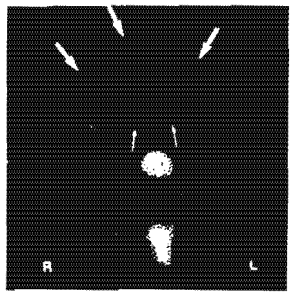

Fig. 1: Transverse slice at the level of OM line + 30 mm from the SPET study of a 11 year old GTS patient with family history of tics and OCD. He presented with OCD, echolalia and no ADD. He had been on treatment with no success and was studied under no medication. This transverse section demonstrates severe bilateral impairment of the perfusion to the cortex of frontal and posterior parietal lobes. In addition, there is marked reduction of the perfusion in the heads of the caudate nuclei, particularly on the left hemisphere.

Conclusion - This study demonstrates that high resolution gamma camera SPET studies identify perfusion deficits in the cortex of frontal, posterior parietal and temporal lobes in patients with Gilles de la Tourette syndrome.

For the first time we have shown perfusion deficits (symmetrical or asymmetrical) in the basal ganglia, particularly the heads of the caudate nuclei, linked to this particular disease (GTS).

These perfusion changes may explain the extrapyramidal and vocal abnormalities found in patients with Gilles de la Tourette syndrome.

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"DOPAMINE D2 RECEPTOR AVAILABILITY IN PATIENTS WITH GILLES DE LA TOURETTE SYNDROME STUDIED WITH SPET"

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Introduction

Patients with Gilles de la Tourette Syndrome (GTS) are afflicted by a movement disorder characterised by involuntary motor tics, vocalisations and, in some patients, coprolalia, copropraxia, echolalia and echopraxia[1]. This wide variety of motor and vocal tics may change over time either in frequency, complexity, severity or anatomical localisation. The onset is usually before the adult life [2]. Some kinds of obsessional disorders also seem to be an integral part of GTS.

With an unknown exact prevalence, GTS has been found worlwide irrespective of culture, racial groups or social classes. It is accepted that 0.5 per thousand of population suffer from GTS, with an estimated total number of 110,000 patients in the U.S.A. and approximately 27,500 in the U.K. [2]. GTS is more frequently found in males than in females with a sex ratio of 4:1.

The exact etiology of GTS is unknown. However, it is now generally accepted as a biological rather than a psychodynamic disorder. Several putative neurotransmitter abnormalities have already been reported [3,4]. Of them, dopamine has been most frequently associated with the disease due to the symptomatic improvement of GTS patients when treated with dopamine D2 receptor blocking agents (e.g. haloperidol and sulpiride). On the contrary, dopaminergic stimulants (e.g. pemoline, methylphenidate) worsen all the symptoms and particularly GTS patients' behaviour.

The dopamine hypothesis to explain GTS etiology suggests either an abnormal concentration of dopamine released from the dopaminergic neurons or a super-sentivity of the dopamine receptors to this neurotransmitter [4].

Objective of the study

We decided to use the new dopamine D2 receptor ligand ^{123}I-iodobenzamide (^{123}I-IBZM) and single slice dynamic single photon emission tomography (Dynamic SPET) [5] to investigate possible changes in the availability of dopamine D2 receptors in the brain of patients with GTS, in comparison with a group of control subjects.

Patient population

This study had the approval of the Ethics Committees of all hospitals involved and ARSAC.

Each individual gave informed written consent before participating on the study. All GTS patients were recruited from the Gilles de la Tourette Syndrome clinic at the National Hospitals for Neurology and Neurosurgery, and clinically evaluated by a neuropsychiatrist (MMR) who has previously reported [6] on the patterns of referral and demographic distribution of GTS patients.

Diagnosis of GTS was established according to the DSM III R criteria.

The control subjects were recruited from hospital staff at the Institute of Psychiatry and clinically assessed by one of the authors (LP).

15 GTS patients were studied and subdivided into 2 groups (Table 1).

The control group consisted of 6 normal volunteers (4 males and 2 females) with a mean age of 30 years. All subjects (controls and GTS patients) were right handed.

TABLE 1 - Clinical characteristics of unmedicated (**UNMED**) and medicated (**MED**) GTS patients.

	Patients with Gilles de la Tourette Syndrome	
	UNMED	**MED**
n	8	7
Mean age	23	24
Age range	10 - 45	12 - 38
M / F	5 / 3	6 / 1
OCD	6	3
ADDH	0	4
Medication	4 naives 4 off (> 90 days)	3 clomipramine + sulpiride 2 flupenthixol 1 haloperidol 1 sulpiride

OCD = Obsessive Compulsive Disorder

ADDH = Attention Deficit Disorder with Hyperactivity

Materials and Methods

Dynamic SPET (Fig. 1) was performed following the protocol previously described [5]. Each individual was positioned supine on the couch of the SME 810 brain tomographic scanner. An intravenous antecubital line was placed in the basilar vein. A basal ganglia transverse plane, parallel to the canto-meatal line and 30 mm above was chosen to coincide with the detection plane of the SME 810.

185 MBq of ^{123}I-IBZM was injected through the antecubital iv line and 5 minute slices were acquired for as long as possible (60 - 90 minutes) according to individual cooperation.

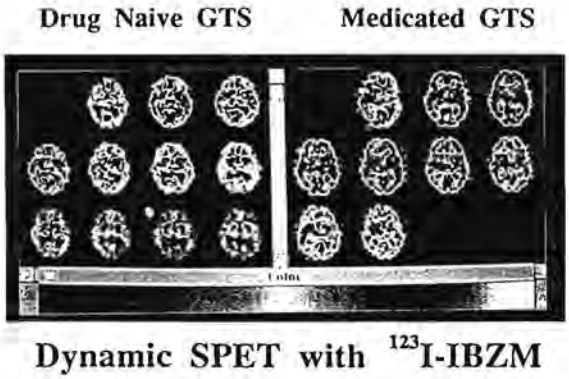
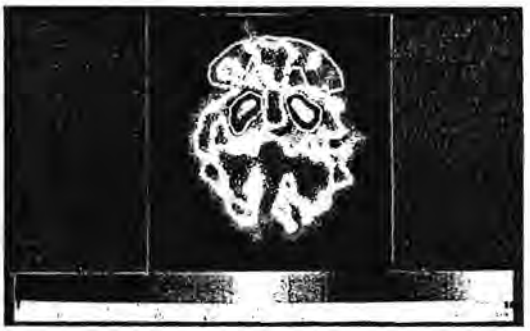


Figure 1. ^{123}I-IBZM kinetics in drug naive GTS differs significantly from medicated GTS patients.

Image processing was performed using manufacturers' software protocols in the Macintosh II computer.

Regions of interest were drawn (Fig. 2) around the basal ganglia (BG) on each hemisphere, using a 70% isocontour, and around the frontal cortex (FC) at the 40% isocontour level. BG over FC ratios of average counts per pixel were calculated and compared between the three groups (UNM GTS, MED GTS and Controls).



ROI analysis

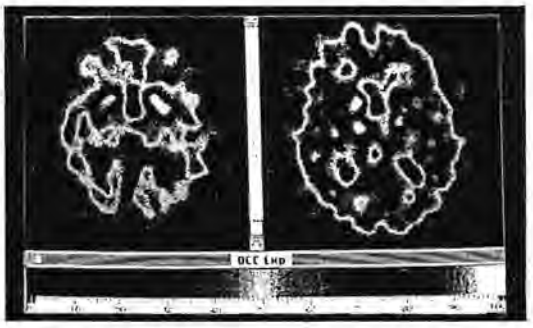
Figure 2. Regions of interest were drawn around the Basal Ganglia and frontal cortex.

Independent two-tailed student t test and ANOVA were used to compare the means of each group. Both statistical methods showed comparative statistical significance.

Results

As expected, medicated GTS subjects taking dopamine D2 receptor blocking drugs showed a marked reduction (Fig. 3) of the ^{123}I-IBZM binding to the BG (bilaterally).

Drug Naive GTS **Medicated GTS**



^{123}I-IBZM 60 minutes post-injection

(single slice 35 mm above the OM line)

Figure 3. The Basal Ganglia are well defined at 60 minutes post-injection in Drug Naive GTS patients. In medicated GTS patients, the distribution of ^{123}I-IBZM is similar to the distribution of a brain perfusion tacer.

This was significantly different from controls from 20 to 60 minutes post-injection (Table 2). This may reflect lower availability of dopamine D2 receptors in the BG.

TABLE 2 - Right and left BG / FC ratios of average counts per pixel.

	Time	Control	UNMED	p	MED	p
Right BG/FC	5	1.22	1.16	-	1.15	-
	10	1.27	1.16	-	1.16	-
	20	1.37	1.23	-	1.22	0.032
	30	1.50	1.33	0.012	1.25	0.000
	40	1.56	1.42	0.082	1.30	0.006
	50	1.64	1.60	-	1.33	0.008
	60	1.77	1.70	-	1.31	0.013
Left BG/FC	5	1.25	1.11	-	1.15	-
	10	1.15	1.13	-	1.19	-
	20	1.30	1.23	-	1.21	-
	30	1.44	1.36	-	1.22	0.014
	40	1.49	1.43	-	1.24	0.001
	50	1.57	1.54	-	1.29	0.016
	60	1.60	1.61	-	1.27	0.033

Unmedicated GTS patients showed ^{123}I-IBZM lower than controls from 5 to 50 minutes post-injection on the BG of both hemispheres (Table 2). However, only at 30 minutes on the left BG and 25 minutes on the right BG, the differences were statistically significant.

A small number of patients has been investigated. Nevertheless two hypothesis may be put forward to explain our findings:

1) these results may be compatible with a diminished initial distribution of ^{123}I-IBZM to the BG, possibly due to perfusion abnormalities, as already reported [7];

2) these findings may suggest a slower binding of the ^{123}I-IBZM to the dopamine D2 receptors in the BG due to a hyperdopaminergic state.

The fact that by 60 minutes post-injection the ^{123}I-IBZM binding in the BG of unmedicated GTS patients is similar to normal controls does not help the distinction between these possible explanations.

These preliminary results need further investigation. Neuroimaging with specific neuroligands and SPET is a promising tool to investigate the pathophysiology of Gilles de la Tourette Syndrome.

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Elevated Frontal Cerebral Blood Flow in Gilles de la Tourette Syndrome: A $^{99}Tc^{m}$-HMPAO SPECT Study

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Abstract. Case reports, numerous brain imaging studies, and certain disease states suggest that the orbital frontal cortex and the striatum are dysfunctional in obsessive-compulsive disorder (OCD). Interest has also grown recently concerning the genetic, neuroanatomic, and clinical links between OCD, chronic motor tics, and Gilles de la Tourette Syndrome (GTS). To test the hypothesis of possible orbito-frontal/basal ganglia dysfunction in GTS, similar to OCD, we studied 20 unmedicated GTS subjects, 10 of whom also had comorbid OCD (GTS/OCD), and 8 control subjects. The subjects were examined with high-resolution single photon emission computed tomography (SPECT) and the labeled regional cerebral blood flow (rCBF) ligand technetium-99m-*d,l*-hexamethyl-propylene amine oxime ($^{99}Tc^{m}$-HMPAO). As a group, GTS subjects showed significantly elevated right frontal/visual cortex activity (mean = 0.879, SD = 0.107) compared with control subjects (mean = 0.798, SD = 0.049). A subanalysis comparing simple GTS versus GTS with comorbid OCD failed to reveal significant differences in regional flow.

Key Words. Single photon emission computed tomography, tic disorder, obsessive-compulsive disorder, orbital frontal cortex, basal ganglia.

Links between Gilles de la Tourette Syndrome (GTS) and obsessive-compulsive disorder (OCD) are intriguing and theoretically very important. Georges Gilles de la Tourette first noted the clinical association between OCD and GTS in his original paper describing the syndrome that now bears his name (Gilles de la Tourette, 1885). Since then, numerous clinicians have noted that GTS patients often have associated OC symptoms (Wasman et al., 1978; Pitman et al., 1987; Bornstein, 1991). From 20% to 60% of GTS patients have comorbid OCD, and their OCD symptoms are

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frequently more disabling than their GTS tics (Robertson et al., 1988). Pauls et al. (1986) showed that family members of patients with either OCD or GTS often have the other disorder. Later, Pauls and Leckman (1986) demonstrated that OCD, chronic motor tic disorder, and GTS are genetically linked, suggesting a common autosomal dominant gene with differing phenotypes. This has led to speculation that GTS may be a useful model for understanding aspects of OCD (Cummings and Frankel, 1985).

Baxter and colleagues (Baxter et al., 1989, 1990; Baxter, 1990) have developed a model of OCD and GTS based on their positron emission tomographic (PET) studies where OCD subjects had increased orbital-frontal and basal ganglia activity compared with control subjects. Their model holds that, depending on the neuronanatomical location of pathology within the basal ganglia, one might express pure OCD, OCD with tics, or GTS. To examine this theory and to determine if a similar regional cerebral blood flow pattern (rCBF) exists in GTS, we studied the relationships between basal ganglia and frontal cortex metabolism in subjects with GTS, subjects with GTS plus OCD, and control subjects. The present study used high-resolution single photon emission computed tomography (SPECT).

Methods

Subjects. The subjects were recruited from the Gilles de la Tourette Syndrome Clinic at the National Hospitals for Neurology and Neurosurgery, Queen Square. The clinic's referral pattern and the demographic makeup of the patient population have been previously described (Robertson, 1989). Approximately 1-2 new GTS patients are evaluated per week from throughout the United Kingdom, with a referral base > 56 million. Subjects were examined by at least two of the authors to ensure that the diagnoses of GTS and OCD were correct. The subjects and were specifically asked whether they had obsessions or compulsions. Additional OC screening included the Yale-Brown Obsessive-Compulsive Scale and the Leyton Obsessional Inventory (Goodman et al., 1989). Control subjects were volunteer medical personnel screened by the authors to be free of present or past psychiatric illness. The GTS and control groups did not significantly differ with respect to mean age (GTS subjects: mean = 23.8 years, SD = 12.02; control subjects: mean age = 34.7 years, SD = 12.5), sex (GTS subjects: 3 women, 17 men; control subjects: 3 women, 5 men), or handedness (GTS subjects: 18/20 right-handed; control subjects, 5/5 right-handed).

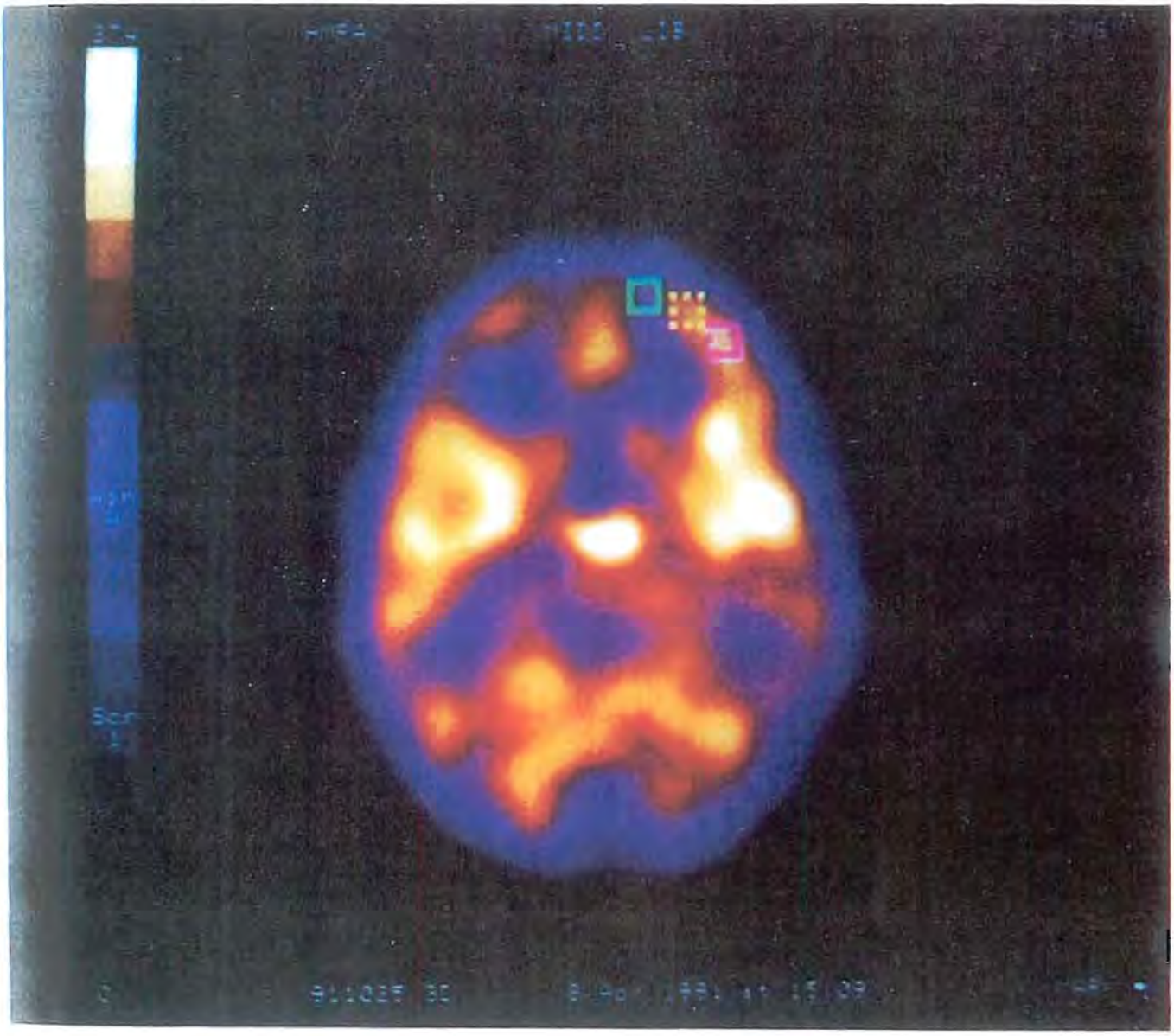
Procedure. The rCBF SPECT scans were performed using a high-resolution (7-9 mm full-width at half-maximum) triple-detector SPECT system (IGE Neurocam) at the Institute of Nuclear Medicine (UCMSM) (George et al., 1991*a*; Townsend et al., 1991). Subjects were given an intravenous injection (average 10 MBq/kg) of technetium-99m-*d,l*-hexamethyl-propylene amine oxime ($^{99}Tc^{m}$-HMPAO) while they were sitting silently with their eyes open in a quiet, lighted room. The radioligand $^{99}Tc^{m}$ -HMPAO is a lipophilic compound that rapidly crosses the blood-brain barrier; within 5 minutes after injection, the radioligand enters the brain, where it becomes hydrophylic and remains stable for hours (Ell et al., 1985). Thus, $^{99}Tc^{m}$ -HMPAO SPECT depicts blood flow, which under most conditions corresponds with brain metabolism (Sokoloff, 1977, 1978).

Approximately 10-30 minutes after the injection, subjects, who were supine, were scanned for approximately 15 minutes (128 projections in a 64 × 64 matrix acquisition) using high-resolution (7-9 mm full-width at half-maximum) collimators. Tomographic slices were then reconstructed in the transaxial, coronal, and sagittal planes using a Hanning prefilter with 0.8 cycles/cm cutoff frequency, a Ramp filter, and attenuation correction. The uptake and activity levels of $^{99}Tc^{m}$ -HMPAO were computed for 4 × 4 nonoverlapping pixel regions of interest (ROIs) of size 1.6 × 1.6 cm^2. ROIs comprised the right and left frontal, medial

temporal, lateral temporal, and occipital lobes, and the right and left basal ganglia and cerebellum (see Fig. 1). The ROI analysis was performed by one of the authors (M.S.G.), who was unaware of the subjects' identities. Nonoverlapping 4 × 4 pixel ROIs were independently placed three times in each region on three different slices, with a neuroanatomic atlas being used for reference. The mean of these nine independent samples was the ROI value for each region (total brain region covered = approximately 2.4 vertical cm). This sampling method has a high intraclass correlation coefficient of 0.92 (1 = total correlation, > 0.70 = acceptable, and 0 = no correlation).

Statistics. Multiple factors can influence an absolute ROI level in a given patient for a given region (e.g., accurate dosing/kg, amount of tracer left in the syringe, tracer deposited subcutaneously and not intravenously). However, ratios of brain activity in each patient can be used to minimize these factors and compare regional values across subjects. The uptake of $^{99}Tc^{m}$-HMPAO in different ROIs was therefore calculated and expressed as a ratio to visual cortex values. We chose the visual cortex as the reference region over the cerebellum, which may be affected in GTS, especially in subjects who might be having, or suppressing, motor tics

Fig. 1. A transverse 99Tcm-HMPAO SPECT scan of a GTS subject at the level of the basal ganglia



Three 4 × 4 pixel regions of interest are shown over the right frontal region, where significant differences were found between GTS and control subjects. $^{99}Tc^{m}$-HMPAO SPECT = single photon emission computed tomography with technetium-99m-*d,l*-hexamethyl-propylene amine oxime as ligand. GTS = Gilles de la Tourette syndrome.

during the injection. Regional values are also expressed as ratios to whole brain uptake. GTS subjects were largely quiet through the injection and scanning process, and were free of tics. No subjects had staring tics. Group means of regional brain-activity ratios were compared between GTS subjects and control subjects by a one-factor analysis of variance (ANOVA) for the following regions: left and right basal ganglia/visual cortex and left and right basal ganglia/whole brain; left, midline, and right frontal cortex/visual cortex and left, midline, and right frontal cortex/whole brain). To test whether age was a factor in the results, a correlational analysis was done with age and left frontal/whole brain and also with left frontal/visual cortex ($R^2 = 0.109$).

Results

GTS subjects had increased right frontal activity compared with control subjects when this region was normalized to the visual cortex (GTS subjects: mean = 0.879, SD = 0.107; control subjects: mean = 0.798, SD = 0.049; $p < 0.05$, ANOVA) (Table 1). The difference was also significant when this region was expressed as a ratio to whole brain activity. A separate ANOVA that compared these same brain ratios between GTS subjects with and without OCD did not demonstrate significant differences.

Table 1. Mean regional cerebral blood flow by regions and groups

	GTS			
Ratio	Simple GTS ($n = 10$)	GTS+ OCD ($n = 10$)	Both ($n = 20$)	Controls ($n = 8$)
Left frontal/visual cortex	0.89 (0.09)	0.89 (0.105)	0.89 (0.095)	0.82 (0.065)
Right frontal/visual cortex	0.87 (0.072)	0.88 (0.138)	0.88 (0.107)[1]	0.79 (0.049)
Midline frontal/visual cortex	1.02 (0.075)	1.02 (0.15)	1.03 (0.115)	0.98 (0.067)
Left basal ganglia/visual cortex	0.95 (0.038)	0.94 (0.095)	0.95 (0.071)	0.93 (0.086)
Right basal ganglia/visual cortex	0.97 (0.08)	0.96 (0.086)	0.97 (0.082)	0.95 (0.089)
Left frontal/whole brain			1.04 (0.06)	1.01 (0.07)
Right frontal/whole brain			1.03 (0.06)[1]	0.98 (0.06)
Midline frontal/whole brain			1.20 (0.06)	1.19 (0.06)
Left basal ganglia/whole brain			1.12 (0.05)	1.12 (0.07)
Right basal ganglia/whole brain			1.14 (0.05)	1.14 (0.07)

Note. GTS = Gilles de la Tourette syndrome. OCD = obsessive-compulsive disorder. Values are means; standard deviations follow in parentheses.

1. Two-group (GTS vs. controls) analysis of variance, right frontal/whole brain visual cortex: $F = 4.142$; $df = 26$; $p < 0.0521$.

Discussion

We have demonstrated elevated right frontal rCBF in unmedicated GTS subjects compared with control subjects. These results should be interpreted with some caution, however. A selection bias toward the most severe cases of GTS and OCD probably exists due to the referral pattern of the clinic. Also, the two groups differed in both age and sex ratios, which could have influenced the results, although the differences are not statistically significant.

We considered whether underlying brain atrophy or damage could possibly influence our results. However, atrophy would likely cause a decrease in rCBF, while we found increased rCBF in GTS subjects compared with our normal control subjects. We are now in the process of definitively excluding this possibility with magnetic resonance imaging of these same subjects. Most patients with GTS have no known underlying brain pathology. A series of 53 computed tomographic scans of GTS patients from this same clinic were uniformly normal (Lees et al., 1984).

Interpreted with caution, however, these results are interesting in light of current models of OCD and GTS. Case reports, neurosurgery results, and previous functional neuroimaging studies have all implicated frontal lobe and basal ganglia pathology in OCD. Numerous case reports have shown that orbital frontal and basal ganglia pathology are associated with OCD (for a review, see George et al., 1992). Frontal tumors have been reported in association with OCD (Brickner et al., 1940; Cambier et al., 1988; Seibyl et al., 1989). Similarly, infarctions and infections of the frontal lobes and basal ganglia have been reported in patients with OCD (Schilder, 1938; Wohlfart et al., 1961; Laplane et al., 1981; McKeon et al., 1984; Swedo et al., 1989; Tonkonogy and Barriera, 1989; Weilburg et al. 1989).

Further evidence of the role of the orbital frontal lobes and basal ganglia in OCD is provided by the results of neurosurgery for OCD. Surgically interrupting the frontally projecting fibers in the cingulum (a procedure known as cingulotomy) can often alleviate OC symptoms (Talairach et al., 1973; Tippin and Henn, 1982; Jenike et al., 1991).

Previous functional neuroimaging studies (PET and SPECT) of OCD have almost uniformly found hypermetabolism of the frontal lobes (Baxter et al., 1989, 1990; Nordahl et al., 1989; Swedo et al., 1989*a*; Machlin et al, 1991; George, 1992). Three PET studies of OCD have shown similar but not identical results. In a study with F-2-deoxy-2-fluoro-*d*-glucose (F2FDG) PET, Baxter et al. (1989) found that 10 OCD subjects had increased absolute metabolic rates in the heads of the caudate nuclei and orbital gyri when compared with control subjects. These findings were later replicated in a medication-free, age- and sex-matched, controlled study with 10 OCD subjects. In this replication study (Baxter et al., 1990), OCD subjects had increased metabolic rates in their orbital gyri when expressed as a ratio to the ipsilateral cerebral hemisphere. A PET study by Nordahl et al. (1989) revealed increased metabolic rates in the orbital gyri of eight OCD subjects relative to the whole brain metabolic rate. Finally, Swedo et al. (1989*b*) described 18 childhood-onset OCD subjects whose scans were compared with those of age- and sex-matched control subjects. The OCD subjects had prefrontal abnormalities, with elevated glucose metabolism in the left orbital frontal, right sensory motor, and bilateral prefrontal and anterior cingulate regions. The right prefrontal and left anterior cingulate regions showed increased relative glucose metabolism. In addition, a significant positive correlation emerged between right orbital glucose metabolic rate and OCD severity.

In contrast, Laplane et al. (1989) reported on eight patients with bilateral basal ganglia lesions and behavior suggesting a "frontal-lobe" syndrome and OCD. PET scans with ^{18}FDG as tracer in seven patients revealed hypometabolism of the pre-

frontal cortex relative to other parts of the brain. These studies suggest that damage to the lentiform nuclei causes prefrontal cortex dysfunction, which leads to OCD.

Using $^{99}Tc^{m}$-HMPAO SPECT, Machlin et al. (1991) found medial frontal and cingulate hypermetabolism in 10 OCD subjects compared with control subjects. In contrast, there has been one case report of decreased right basal ganglia and right anterior temporal lobe activity in OCD on the basis of ^{123}I-amphetamine SPECT scans. These abnormalities resolved with pharmacologic treatment (Hamlin et al., 1989).

Recently Swedo et al. (1991) studied 10 female subjects with trichotillomania, a disorder of compulsive hair pulling, whose ^{18}FDG PET scans were compared with those of 20 age- and sex-matched control subjects. Results in this study were very different from those reported by others investigating OCD. They discovered increased blood flow in parietal and cerebellar regions, but not in the frontal lobes or basal ganglia. From this one study, it appears that trichotillomania, often referred to as an OC-spectrum disorder (George et al., 1990; George, 1991), may be associated with a different regional metabolic pattern than OCD.

In summary, most PET and SPECT studies have found a surprisingly similar picture of elevated right frontal metabolism in OCD. The one study in an OCD-related disorder (trichotillomania) found a different rCBF map. Previous SPECT work in GTS is limited, although some have reported qualitative asymmetries in basal ganglia metabolism (Hall et al., 1990). Our results indicate that GTS subjects, similar to subjects with OCD, have elevated right frontal activity compared with control subjects. These findings may be due to the presence in the study group of comorbid OCD/GTS subjects, although the power for this analysis is limited. No basal ganglia differences were observed in GTS, with or without OCD, or in comparisons of either of these groups with control subjects.

Our hypothesis before the study began was that the rCBF picture of GTS versus GTS/OCD subjects would vary, in a manner similar to that postulated by Baxter (1990), with elevated orbital frontal activity in those subjects with OCD and elevated basal ganglia activity in "pure" GTS. We discovered, however, that the entire group of GTS subjects had elevated frontal activity and that there were no statistically significant differences between subgroups of GTS subjects with and without OCD. A previous trend reported by our group (George et al., 1991) continues, with subjects with OCD/GTS having consistently elevated frontal and decreased basal ganglia activity compared with subjects with simple GTS without OCD. These trends, however, failed to reach statistical significance.

The elevated right frontal activity in these GTS subjects strengthens the previous biological associations between GTS and OCD. Our failure to find regional differences between subgroups of subjects with or without OCD does not contradict previous models. Rather, in view of the small number of subjects in this study, and the limited spatial resolution of current SPECT scanners, there is a high likelihood of a type II error (i.e., a difference existed but could not be detected).

Further studies are clearly needed to confirm, extend, and refine these findings. We are now using $^{99}Tc^{m}$-HMPAO SPECT to study "pure" OCD subjects (without tics). Additional SPECT studies using specific neurotransmitter ligands (dopamine

and serotonin) or specific activation protocols (George et al, 1991; Ring et al., 1991) may help to unravel links between OCD and GTS.

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Brain Perfusion Abnormalities in Gilles de la Tourette's Syndrome

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Background. Functional brain imaging with technetium-99m d,l-hexamethyl propyleneamine oxime (HMPAO) Single Photon Emission Tomography (SPET) allows us to explore the cerebral pathophysiology of Gilles de la Tourette's Syndrome (GTS).
Method. Fifty patients and 20 controls were examined. Patients were rated for tic severity and mood. Scans were analysed quantitatively using internal ratios to the occipital cortex.
Results. Patients differed from controls on measures of relative blood flow to the left caudate, anterior cingulate cortex and the left dorsolateral prefrontal cortex. Severity of tics was related to hypoperfusion of the left caudate and cingulate and a left medial temporal region. Hypoperfusion in the left dorsolateral prefrontal region was related to mood.
Conclusions. The areas found to be hypoperfused in this study are consistent with known functions of fronto-striatal circuits. A wide range of perfusion patterns is seen, however, and no characteristic patterns for behavioural subgroups has been documented with this technique.

Patients with Gilles de la Tourette's syndrome (GTS) have a wide range of symptoms. The central feature of the condition is the presence from childhood of multiple motor and vocal tics (American Psychiatric Association, 1987). Other characteristic signs, not essential for the diagnosis, are coprophenomena, echophenomena and palilalia.

The aim of this study was to document the regional cerebral blood flow correlates of GTS and its component symptoms. Firstly, we wished to determine if there were any fundamental differences in perfusion patterns between our patients and a group of normal controls. Secondly, we wanted to examine whether specific patterns of regional blood flow were correlated with the presence of obsessive–compulsive behaviours (OCB), echolalia, coprolalia, echopraxia, copropraxia, treatment, tic severity, mood and demographic characteristics.

Method

Consecutive patients referred to the GTS clinic at the National Hospitals for Neurology and Neurosurgery were asked to have a SPET scan. Women of child-bearing age who were not using adequate contraception were excluded. The project was approved by the ethical committee of the National Hospitals for Neurology and Neurosurgery and the Administration of Radioactive Substances Advisory Committee. All subjects gave informed consent. In all, 50 patients and 20 normal controls were scanned over three years.

Diagnosis of GTS according to DSM-III-R criteria (American Psychiatric Association, 1987) was confirmed by one of the authors (MMR), who also questioned patients about the presence or absence of coincident OCB (enquiring specifically into the presence or absence of obsessive thoughts, forced touching, arithmomania, evening-up and compulsive rituals). Also documented was the presence or absence of echolalia, echopraxia, coprolalia and copropraxia. Tic severity at the time of the scan was rated on a 3-point scale (1 = minimal or mild, 2 = moderate, 3 = severe) (more elaborate rating scales are now in use in our clinic but were not when the early scans were being acquired). The Beck Depression Inventory (BDI), a self-rating scale for depression, was completed by 42 patients (Beck *et al*, 1961).

The study group comprised 38 males and 12 females with a mean age of 24 years (range 7–65); 16 patients were rated as mild, 21 moderate and 13 severe; 27 had significant OCB. Only one of the patients had an IQ less than 80; a further nine were in the range 80–100. Only three had any neurological abnormalities, these being minor 'soft' signs. Of other symptoms, 21 patients had echolalia, 22 echopraxia, 18 coprolalia and 10 copropraxia. BDI scores ranged from 1 to 39 with a mean of 12. Thirty-four patients were drug-free, of whom 24 were drug naïve (never taken drugs). Patients on medication were receiving neuroleptics, mostly low-dose sulpiride; no other medications were being taken. The scans were compared with those obtained from normal volunteers.

Of the volunteers, nine were male and 11 female, with an average age of 23 years. None suffered from

physical or psychiatric illness, and all were drug-free.

Two distinct scanning technologies were used over the three years, the design and performance characteristics of which have been described previously. The Starcam single-headed gamma camera was used for scanning 33 patients (Costa *et al*, 1988) and the GE Neurocam triple-headed brain-dedicated camera for 17 (Kouris *et al*, 1992). All controls were scanned on the Neurocam. Subjects were injected with 550 MBq HMPAO at rest with their eyes open, and scanned for a maximum of 30 min. Ambient light conditions were artificial and the same for all scans. Some patients who could not tolerate this length of time without disruptive tics were scanned for a shorter period. All scans were reconstructed and analysed by the same investigator on the Star 4000 computer. Reconstructions used a filtered back-projection technique with Hanning pre-filter and attenuation correction as described previously (Costa *et al*, 1988). These were in three planes with a final slice thickness of 2 pixels. Transaxial images were reconstructed to include a line drawn from the base of the occipital to the base of the frontal cortex on a midline sagittal slice. Sagittal planes were those parallel to the midline and coronal planes were perpendicular to the axial and sagittal.

Scans were analysed using a region of interest (ROI) method. Regular ROIs were placed manually in 15 regions. For Neurocam scans these were 4×4 pixels (voxel size $1.6 \times 1.6 \times 0.8$ cm) and for Starcam scans 6×6 pixels (voxel size $2 \times 2 \times 0.6$ cm). We chose the visual cortex as our reference region, both because of the reliability of measurements in this region and because we felt that it is unlikely to be involved in the pathophysiology of GTS (unlike the cerebellum).

The brain regions analysed were as follows:

(a) in transverse images: right and left anterior striatum at the level of the thalamus, cingulum and visual cortex (as reference area) at the same level, three cerebellar regions (right, left and midline) at the level where the inferior poles of the temporal lobes are first seen.

(b) in coronal images: the cingulum measured in the slice immediately posterior to that containing the cingulum in its caudal direction, orbital frontal cortex bilaterally, dorsolateral prefrontal region bilaterally in the same slice at the same level lateral to the cingulum, and anterior and posterior medial temporal regions bilaterally, the former immediately anterior to the slice containing the temporal lobes in their greatest width and the latter at the slice containing the brainstem, two slices posterior to this. The measure for the cingulum was taken as the average of the coronal and the horizontal readings.

All ROIs were expressed as within-subject ratios to the visual cortex (Table 1). No attempt was made to compare counts in ROIs directly. The variability of final counts between subjects precludes such comparisons because of inter-subject differences in uptake and retention of HMPAO. These differences depend on the exact dose administered, bodyweight and carotid blood flow as well as cardiac output. The investigator analysing the scans (JM) did not know which scan belonged to which patient and so did not know the specific clinical details of the patients. For those patients scanned on the Starcam, it was not possible to be blind to whether subjects were patients or controls nor, obviously, to which camera was used.

The normal distribution of the ratios for each region individually, in both patients and controls, was established using the Kolmogarov-Smirnov tests for normality. The first analysis looked at the differences between the GTS patients and controls using a 2-tailed *t*-test. The Bonferroni correction for multiple comparisons was applied. We then performed a one-way analysis of variance for all regions across the three groups divided according to tic severity. To examine the effect of depression on SPET blood flow pattern, we subdivided the group according to their score on the BDI. We determined two subgroups of our sample: first, those with BDI scores less than 10 ($n = 19$); and second, those with scores greater than 20 ($n = 8$). Patients with scores between 10 and 20 were excluded from further analysis. A one-way analysis of variance for all regions across the three groups (BDI < 10, BDI > 20 and controls) was then performed. A similar analysis of variance was applied across the groups divided according to medication status.

A modified least significant differences test of significance was applied. This corrects for multiple comparisons.

Pearson's correlations (r) were calculated for each region and our measure of tic severity, and for each region and the BDI score. Finally, we looked within the GTS patient group for any differences between those patients with and without OCB, echolalia, echopraxia, coprolalia or copropraxia. In addition, we looked for any differences between patients of different sex and between those whose scans were performed on the Neurocam and those whose scans were performed on the Starcam gamma camera system.

SPSS/PC+ computer programme (v. 4).

Results

All ratios were normally distributed. The mean values and s.d. of relative blood flow for all 14 areas in patients and controls are given in Table 1. The values are lower for the patient group in both caudate nuclei, the anterior cingulate, both cerebellar hemispheres and the left dorsolateral prefrontal cortex; and higher in the right posterior medial temporal area. There are no differences for the midline cerebellum (vermis), right dorsolateral prefrontal cortex, bilateral orbitofrontal cortex, bilateral anterior medial temporal cortex and left posterior temporal cortex. The left caudate and anterior cingulate survive strict Bonferroni correction. The reduction in the left dorsolateral prefrontal area ($P = 0.007$ before correction) shows a trend ($P = 0.098$) after correction. The distribution of values is wide, both in normal controls and in patients.

We found that the only region where there are any between-group differences for the patients of different degrees of severity was in the left anterior temporal region. The difference is significant

Table 1
Mean values (s.d.) for each of 14 regions expressed as ratio to visual cortex

Region	GTS	Controls	P
Right caudate	0.84 (0.08)	0.89 (0.07)	*
Left caudate	0.85 (0.07)	0.92 (0.07)	****
Cingulum	0.89 (0.06)	0.94 (0.06)	***
Right cerebellum	0.99 (0.08)	1.04 (0.08)	*
Left cerebellum	0.98 (0.07)	1.02 (0.09)	*
Midline cerebellum	0.97 (0.07)	0.97 (0.08)	
Right dorsolateral prefrontal	0.79 (0.06)	0.81 (0.07)	
Left dorsolateral prefrontal	0.77 (0.08)	0.83 (0.08)	**
Right orbital frontal	0.72 (0.07)	0.74 (0.05)	
Left orbital frontal	0.71 (0.09)	0.74 (0.06)	
Right anterior medial temporal	0.75 (0.06)	0.76 (0.05)	
Left anterior medial temporal	0.77 (0.07)	0.79 (0.06)	
Right posterior medial temporal	0.80 (0.08)	0.77 (0.05)	*
Left posterior medial temporal	0.80 (0.07)	0.81 (0.07)	

s.d. = Standard deviation
Results of unpaired *t*-test comparison of group means:
*$0.01 < P < 0.05$
**$0.005 < P < 0.01$
***$0.001 < P < 0.005$
****$P < 0.001$

s.d. 0.06) and those rated as moderate (0.75, s.d. 0.06).

Dividing the patients according to BDI score showed there to be significant between-group differences for blood flow in several regions: the right and left cerebellum, the left caudate, the cingulum and the left dorsolateral prefrontal cortex. For the cerebellar areas, the caudate and the cingulum, the differences were only significant at the 5% level between controls and patients with a low BDI score. The means of the low and high BDI score patients are, however, comparable for the caudate and cingulum, whereas for the cerebellar areas, the more depressed GTS patients have cerebral blood flow ratios comparable to those of the control group (see Table 2). The differences for the dorsolateral prefrontal area are, however, more marked in (and significant for) the high BDI score patients.

The Pearson's correlations are shown in Table 3. The strongest correlation for tic score was with the left caudate and cingulum, followed by the left dorsolateral prefrontal and left anterior temporal. These were all inverse correlations. The BDI score showed an inverse correlation with left dorsolateral prefrontal perfusion.

The patients on medication at the time of the scan had higher blood flow than those not on medication (drug-free or drug-naïve). These differences were not significant.

Table 2
Summary of means (s.d.) for regions showing between group differences for low and high Beck score patients and for controls

ROI	Low Beck score ($n = 19$)	High Beck score ($n = 8$)	Controls ($n = 20$)
Right cerebellum	0.97 (0.08)*	1.01 (0.05)	1.04 (0.08)
Left cerebellum	0.94 (0.06)*	1.00 (0.08)	1.02 (0.09)
Left caudate	0.83 (0.07)*	0.86 (0.04)	0.92 (0.07)
Cingulum	0.88 (0.05)*	0.89 (0.06)	0.94 (0.06
Left dorsolateral prefrontal	0.79 (0.09)	0.74 (0.07)*	0.83 (0.08

*indicates difference from normal controls significant ($P < 0.05$) after correction for multiple comparisons.

Table 3
Correlations between regions and measures of tic severity and Beck score

Variables	Regions	r
Tic severity	Left caudate	−0.4
	Cingulum	−0.3
	Left dorsolateral prefrontal	−0.3
	Left anterior temporal	−0.3
BDI score	Left dorsolateral prefrontal	−0.3

There were some further tendencies of interest. Because of the number of variables involved, these differences were not significant after correction for multiple comparisons. Males had similar or lower blood flow than females in all regions except the dorsolateral prefrontal regions bilaterally. Patients with OCB had lower perfusion in all regions than those without OCB; 58% of the males and 41% of the females had OCB and the mean BDI score was 10 in males and 15 in females, but these differences are not significant (χ^2 and t-test). Patients with echolalia, echopraxia, coprolalia or copropraxia could not be distinguished and there were no differences attributable to the type of scanner used.

Discussion

The central feature of GTS is the occurrence of tics. It has been agreed that the diagnosis should be confined to those with both motor and vocal tics (though these need not necessarily have been concurrent). In addition, the disorder has been associated with a range of pathological behaviours such as OCB and self-injury (Robertson, 1989).

Structural imaging studies of GTS have, until very recently, been disappointing. More recently, volumetric magnetic resonance imaging techniques (Peterson *et al*, 1993; Singer *et al*, 1993) have suggested a reduction in volume of the left lenticular region (putamen and globus pallidus) in patients with GTS compared to controls with some loss of the usual left predominant anatomical asymmetry.

One group has reported a series of PET scans of GTS subjects. They have described abnormal associations between metabolic rates in sensorimotor cortex and limbic areas (Stoetter *et al*, 1992). A single report of HMPAO brain scans in a series of nine adults with GTS found left striatal hypoperfusion (Riddle *et al*, 1992). A preliminary report from Dimitsopulos *et al* (1993) of six patients showed reduction in perfusion of the non-dominant basal ganglia. Existing studies thus support the hypothesis (Leckman *et al*, 1991) that the basal ganglia are involved in the pathogenesis of GTS.

There are a number of methodological problems with our study. Our GTS population and controls are not matched for age or sex, and our GTS population was studied with two quite different scanning technologies. The first of these problems results to a large degree from the ethical problems associated with the administration of radiotracers to normal populations, which obliged us to use existing data collected for the newer scanner (Neurocam). If we were to limit our patient group to the same age range (young adults), our sample size would be prohibitively small.

The second problem also relates to sample size. We aimed in this study to examine the spread and pattern of perfusion abnormalities seen in patients with GTS. The approach we took is supported by our finding that there were no differences between the results obtained for our Tourette patients with the two different scanners. We chose the box ROIs described in order to get the best agreement for voxel size between the two methods of measurement.

Finally, we cannot exclude the possibility of a greater partial volume effect occurring in our patient group because of the scanner used and because of a greater degree of head movement expected in the GTS group. Again, however, we would expect such systematic error to result in differences within our patient group according to which scanner was used, and it would not explain the specificity of our findings for any particular structure. Such an error would be likely to have contributed to the large variance in our measurements.

We believe that, in the absence of contemporaneous MRI images for co-registration, our ROI technique maximises the validity of the measurements at some cost to reliability. The latter can probably be improved by the use of anatomical templates. This would then involve the assumption that all our subjects' scans could be analysed as if their brains were of identical shape and size. Indeed, what is as striking as our findings of regional differences between our patient and control groups, is the considerable variations we found, not only among patients but also among normal subjects. It is likely that subtle differences of cerebral perfusion which might correlate with the range of behavioural abnormalities seen in this condition could be lost in the within-group variability.

Notwithstanding these methodological reservations, we found the left caudate to be the area showing the most marked hypoperfusion in our patients. The striatum is involved in the initiation and execution of movement (Montgomery & Buchholz, 1991). Ventral striatal areas are also involved in wider behavioural functions. Moreover, the left caudate has consistently been more deeply implicated than the right in the development of depression (Starkstein *et al*, 1988). The reduced caudate perfusion we find in GTS is consistent with the motor and affective functions identified in this structure. The resolution of these scans does not allow us to differentiate reliably between caudate and lenticular perfusion.

We also found that the anterior cingulate and left dorsolateral prefrontal cortices showed hypoperfusion in the GTS patients. These areas, along with the orbitofrontal cortex, are the cortical areas where the

The dorsolateral prefrontal cortex projects primarily to the dorsolateral head of the caudate nucleus, and the projections from here are to the dorsomedial globus pallidus interna and rostral substantia nigra. Pallidal and nigral neurones then project to the ventral anterior and medial dorsal thalamic nuclei, which in turn connect back to the dorsolateral prefrontal cortex (Alexander *et al*, 1986). Deficits in motor programming, as evidenced by impaired performance on alternating and reciprocal motor tasks, characterise lesions in this circuit (Cummings, 1993). These deficits have recently been documented by our group in GTS.

Our finding that the left anterior temporal region is the only region showing between-group differences in our analysis of variance across groups of differing severity, and the (admittedly weak) correlation between this area and our measure of tic severity, are intriguing as it has been elegantly argued that medial temporal structures, and the amygdala in particular, may play a crucial role in the pathogenesis of GTS (Jadresic, 1992). The natural history of this disorder is such that it will tend to wax and wane and its 'severity' will reflect a complex interaction of the tics themselves, the associated behavioural disturbances and the distress and dysthymia these may evoke in the patient. This makes the interpretation of this finding particularly difficult.

Our finding that the dorsolateral prefrontal hypoperfusion is most marked for the patients with high BDI score, and the correlation between perfusion of this area and the BDI score, suggest that hypoperfusion of this area may be related to affect as much as to diagnosis. This is consistent with other functional imaging studies of depressive illness (Bench *et al*, 1992).

The anterior cingulate cortex has significant projections to the ventral striatum and forms part of a distinct circuit from here to a rostrolateral sector of the internal pallidal segment, which projects in turn to a paramedian portion of the mediodorsal nucleus of the thalamus. Posterior and medial portions of the medial dorsal nucleus project to the cingulate (Alexander *et al*, 1986). Stimulation of this area of the cortex is associated with the generation of involuntary vocalisations (Jurgens, 1976) and cingulate epilepsy is associated with complex motor automatisms at onset of the seizure. Our finding of hypoperfusion of this area in a disorder characterised by involuntary vocalisations and motor activity is thus intriguing.

Some degree of consensus has emerged in the literature regarding the finding of hyperperfusion of *al*, 1992) of 20 patients with GTS, including 10 with coincident OCD, showed increased right orbitofrontal blood flow compared to a group of eight normal controls. (These patients were not part of the present study.) This earlier study did not include a correction for multiple comparisons, and the frontal hyperperfusion was relative to an unusually low measure of perfusion in the controls. It is perhaps not surprising that we did not find such hyperperfusion in our GTS patients with OCB. The obsessive characteristics of patients with GTS are different from those seen in OCD (George *et al* 1993). Frontal hyperperfusion may be more a feature of increased arousal and anxiety, which is more prominent and more frequent in OCD than in GTS with OCB.

The cerebellum has been implicated in quite a range of cognitive processes, as well as playing a key role in motor coordination. It has been shown to be involved in the imagining of movement and not just in movement itself (Decety *et al*, 1990). We find relative hypoperfusion of this area in GTS. Within the patient group, greater perfusion in the cerebellar hemispheres in the more depressed GTS patients is consistent with the findings described by Bench *et al* (1992) in a PET study of primary depressive illness.

The mean BDI score was higher in our female than in our male patients. Although this difference was not statistically significant, it may contribute to the finding that the blood flow in dorsolateral prefrontal regions in women is relatively lower than in men.

Essentially, we have shown that there is hypoperfusion of certain brain areas in patients with GTS. These areas are consistent with previously proposed cortico-striatal-thalamo-cortical (CSTC) circuit abnormalities in GTS. The clinical variability of the disorder is likely to reflect differential involvement of circuits subserving motor, affective and volitional aspects of behaviour. The nice dissection of these discrete but interlinked circuits remains beyond the scope of present SPET techniques, but studies are under way using PET technologies.

Further research should now also be directed at determining the clinical significance of the present findings and, in particular, whether the finding of cerebral hypoperfusion has prognostic significance.

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BRIEF COMMUNICATION

HMPAO SPET does not distinguish obsessive–compulsive and tic syndromes in families multiply affected with Gilles de la Tourette's syndrome

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ABSTRACT

Background. Gilles de la Tourette's syndrome (GTS) is a familial neuropsychiatric disorder characterized by tics and obsessive–compulsive behaviours (OCB). Previous HMPAO SPET studies of subjects with GTS have shown hypoperfusion of striatal and frontal areas. Studies of patients with primary obsessive–compulsive disorder have shown, in contrast, hyperperfusion of similar areas.

Methods. Twenty subjects from five families affected by GTS, including individuals with OCB but no tics, were examined using HMPAO SPET.

Results. There were abnormalities of regional cerebral perfusion in individuals with GTS, OCB and tics. Hypoperfusion was in striatal, frontal and temporal areas. There was no hyperperfusion.

Conclusions. Regional cerebral blood flow patterns in individuals with OCB in families affected by GTS are comparable to their relatives with GTS and differ from individuals with primary OCD in the absence of a family history of tic disorders.

INTRODUCTION

Functional neuroimaging offers the possibility of a deeper understanding of the physiology of behaviour. Images of regional cerebral blood flow or metabolism in patients with neuropsychiatric disorders help us to describe the neurology of conditions that can currently only be described in behavioural terms.

GTS is a neuropsychiatric disorder defined by the presence of multiple motor and vocal tics (American Psychiatric Association, 1987). GTS is associated with more complex abnormal behaviours involving imitation and profanation. Echolalia, echopraxia, coprolalia and copropraxia need not be present to make a diagnosis of GTS, but are characteristic of the disorder. OCB are also associated with the condition, as is attention deficit and hyperactivity (Robertson, 1994). Genetic (Pauls & Leckman, 1986; Eapen *et al.* 1993) and epidemiological (Myers *et al.* 1984; Robins *et al.* 1984) studies suggest that for OCB at least, the association is biological. There are similarities of phenomenology between tics, obsessions and compulsions that suggest a shared pathophysiology.

Functional imaging studies of GTS and OCD have thrown some light on the brain substrates of these syndromes. Although no definitive

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abnormalities have been found there is a tentative consensus as to the brain areas involved. Particularly implicated are striatal and frontal areas. OCD has been associated with hyperperfusion of frontal areas (Nordahl *et al.* 1989; Baxter, 1990; Benkelfat *et al.* 1990; Machlin *et al.* 1991; Swedo *et al.* 1992). The available literature on perfusion patterns or abnormalities in GTS is limited. Preliminary studies by Riddle *et al.* (1992), Kurlan's group (Dimitsopulos *et al.* 1993) and our own series (Moriarty *et al.* 1995) suggest involvement of caudate, anterior cingulate, medial temporal and dorsolateral prefrontal areas. In all studies the abnormalities are in the form of hypoperfusion rather than increased blood flow.

Thus, OCB and GTS have apparent genetic and biological associations but conflicting cerebral blood flow findings. We explored the patterns of cerebral blood flow seen in families containing subjects with GTS and OCB. Specifically, we wished to test the hypothesis that patients with OCB could be distinguished from patients with GTS by SPET. We chose to use the term OCB rather than OCD in this context to refer to subjects who fulfilled the symptoms criteria of the DSM-III-R (American Psychiatric Association, 1987) diagnosis of OCD but where these symptoms did not necessarily interfere with the social and occupational functioning of the individual.

METHOD

Five families with at least one child affected by GTS were identified. These were selected by one investigator (V.E.) to include specifically a spectrum of disorder comprising GTS, OCB and unaffected family members. Families consisted of two parents and two children. Direct clinical examination was carried out on all subjects, using the National Hospital Schedule for tics and related disorders. This instrument has been described previously (Eapen *et al.* 1993). In addition, each family member was asked independently about the other members of his/her family. For those subjects reported by family members as having tics or OCB, we established this on direct clinical interview. Definite diagnosis of tics or OCB was made if symptoms were present both on history and on examination. The diagnosis of GTS was made according to DSM-III-R criteria (American Psychiatric Association, 1987). In all, there were eight subjects with GTS, four with OCB, two with tics (that did not fulfil the criteria for GTS) and seven subjects were unaffected. One subject had OCB and tics, but not GTS.

Scans were acquired following the routine protocol of the Institute of Nuclear Medicine (INM). All subjects were studied with the GE Neurocam triple-headed brain dedicated gamma camera (Kouris *et al.* 1992) after injection with 550 MBq of ^{99m}Tc hexamethylpropylene amine oxime (HMPAO) at rest with eyes closed. Data processing and analysis were performed on a Star 4000 computer (Costa *et al.* 1988). Three orientation planes with a final slice thickness of 2 pixels were studied. All scans were read by D.C.C. who was blind to the subjects' clinical status. The scans were reported qualitatively and quantitative radioactivity ratios (cortical/cerebellum) were calculated. Normality was defined relative to a control database following the protocol at the INM (Costa *et al.* 1993).

All patients were rated at the time of the scan for degree of anxiety using a visual analogue scale.

After a complete description of the study, written informed consent was obtained from all adult subjects and from the parents of children, with the child's assent. None of the subjects had been scanned previously. The subjects with GTS were not part of the cohort we have previously reported (Moriarty *et al.* 1995).

RESULTS

Detailed results are presented in the Appendix 1, the subjects are grouped according to family (A to E). None of the seven family members who were entirely symptom free had abnormalities of their SPET scans. Of the 13 symptomatic subjects, only three had normal SPET scans and nine were abnormal (Fisher's Exact Test, two-tailed, $P = 0{\cdot}0031$). One scan, in an 8-year-old boy with GTS (A4) was obscured by movement artefact. Of the seven subjects with GTS who had good quality scans, hypoperfusion of the caudate nucleus (on either side or bilaterally) was seen in five, parietal or temporal in five, thalamus in two, frontal in one and brainstem in

one. Frontal, parietal, temporal and caudate involvement was seen in the three subjects with OCB. No hyperperfusion was reported in any of the scans.

Affected subjects, including those with OCB, scored lower than the unaffected family members on the visual analogue anxiety scale. (Patients 1·6, S.D. = 1.4; normals 3·1, S.D. 2·2.) This is not a statistically significant difference.

DISCUSSION

Perfusion in GTS subjects

The SPET findings of a variable pattern of hypoperfusion involving frontal, striatal and temporal areas in patients with GTS which has been reported by ourselves and others (Riddle *et al.* 1992; Dimitsopulos *et al.* 1993; Moriarty *et al.* 1995) is confirmed by this study. Unaffected family members had normal scans. Normal scans in clinically symptomatic subjects were seen in both patients with tics alone but without GTS itself and in the one subject with OCB and tics but not GTS. This suggests that whatever the perfusion abnormalities seen in patients with GTS they are not a reflection of the tics *per se*. They may be related to severity (this was not formally rated as part of this study) or reflect the complexity of the affective, cognitive and motor abnormalities which may be seen in patients with GTS.

Perfusion in subjects with OCB

The scan of the subject with tics and OCB was suboptimal and it cannot be unequivocally described as normal. None of our four subjects with OCB showed increased cerebral perfusion, although one had a normal scan. The others showed areas of decreased perfusion similar to those seen in the patients with GTS. Although it is unlikely that symptoms would be missed both on history and on clinical examination, it is still possible that in some of the younger subjects, symptoms may not yet have been expressed. It is possible that our subjects with OCB represented a less severe population than those subjects reported elsewhere in the literature. While this would possibly account for us not finding the frontal hyperperfusion described in patients with OCD, it cannot account for our finding of abnormal hypoperfusion. Further studies of patients with OCB matched for severity, with and without family histories of tic disorders would clarify this issue.

In our efforts to control for anxiety, we observed that all affected subjects, including those with OCB, scored lower than unaffected on the visual analogue anxiety scale. Although this is not a statistically significant difference, we believe it reflects a clinical difference between patients with GTS (including OCB) and primary OCD which can be ignored in a literature bent on emphasizing similarity. Patients with GTS, unlike those with OCD, are not distressed by many of their ruminations. Neither are they plagued with the obsessional's fear of acting out their ritualistic ideas. Indeed, it is arguable that patients with GTS are at the opposite pole to those with OCD in that their scatological ideas are not resisted and associated with marked anxiety, but are vented, any resultant anxiety being associated with the embarrassment caused rather than the dislike of the thoughts themselves. Further evidence for the validity of this distinction comes from the study of OCD by Swedo *et al.* (1992), in which orbitofrontal hyperperfusion was shown to have a positive correlation with global anxiety when patients were treated with selective serotonin reuptake inhibitors (SSRIs).

Subgroups of OCB

McDougle *et al.* (1994) performed a double-blind placebo controlled trial of haloperidol as add-on therapy for patients with OCD refractory to treatment with fluvoxamine. Treatment was of benefit in patients who had a co-morbid tic disorder. However, McDougles's study did not distinguish patients without a co-morbid diagnosis of a tic disorder but with a family history of a tic disorder from those without a family history (although it is stated in the discussion that none of the three responders to haloperidol without a co-morbid tic disorder had a known family history of tics). If the relevant distinction were between those with a genetic predisposition towards tic disorders (including a variant of OCD), and those with no family history of tic disorders, this would be consistent with our finding of regional cerebral hypoperfusion in our selected patients with OCD in contrast to the usual finding of hyperperfusion in patients with OCD described in the literature.

CONCLUSION

We propose that our patients with OCB represent a specific subgroup of all patients with OCB, namely those patients with a personal or family history of tics. Present SPET methodology cannot distinguish between different symptomatic subgroups (i.e. patients with OCB from patients with tics alone). Our findings suggest that OCB patients from families affected by tic disorders differ from patients with OCB without such family histories in being less anxious, and being characterized by reduced cerebral perfusion.

APPENDIX

Subject	Age and anxiety	Diagnosis	Anxiety rating	Scan findings
A1	43 M	OCB	1	↓B PAR, ↓L, FR, ↓R, TMP
A2	39 F	Tics	1	Normal
A3	6 F	Nil	2	Normal
A4	8 M	GTS	5	Obscured by artefact
B1	11 M	OCB	1	↓B PAR
B2	15 M	GTS	3	↓B PAR ↓R CN
B3	47 F	Nil	2	Normal
B4	57 M	GTS	1	↓B PAR ↓B FR ↓B THA ↓L TMP ↓BS
C1	49 F	Nil	2	Normal
C2	15 M	GTS	3	Normal
C3	13 F	Nil	5	Normal
C4	55 M	GTS	2	↓B PAR ↓B TMP ↓B CN
D1	10 M	GTS	2	↓B CN
D2	43 F	OCB	0	↓B CN
D3	43 M	Nil	0	Normal
D4	20 F	Nil	6	Normal
E1	45 F	OCB and Tics	2	Normal ~
E2	12 M	GTS	0	↓B CN ↓R TMP
E3	46 M	GTS	0	↓L PAR ↓R TMP ↓B CN ↓R THA
E4	9 M	Nil	5	Normal

↓, Hypoperfusion (compared to control database of INM); ~ due to a degree of claustrophobia in this subject, this scan excluded some of the inferior and anterior parts of the temporal lobes bilaterally.

BS, Brainstem; CN, Caudate; FR, Frontal; PAR, Parietal; THA, Thalamus; TMP, Temporal; B, Bilateral; R, Right; L, Left.

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Dopamine Receptor Availability in Tourette's Syndrome

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Abstract. A large body of evidence suggests that abnormal dopaminergic activity is present in Gilles de la Tourette Syndrome (GTS). To investigate whether dopamine dysregulation involving the D_2/D_3 receptor occurs in GTS, we performed single slice dynamic single photon emission computed tomography (SPECT) with ^{123}iodo-6-methoxybenzamide (^{123}I-IBZM) in 15 GTS patients (eight unmedicated) and six healthy volunteers. After intravenous administration of 5 mCi (185 MBq) of ^{123}I-IBZM, dynamic SPECT (5 minutes per slice) studies were performed at the level of the basal ganglia for 55 minutes. The mean activity per pixel in the basal ganglia was compared with the mean activity per pixel in the visual cortex. Unmedicated GTS patients showed no differences from control subjects. However, GTS patients taking D_2 blocking medications had significantly decreased ^{123}I-IBZM binding compared with control subjects in both the right and left basal ganglia. Thus, D_2/D_3 receptor availability, as measured by ^{123}I-IBZM SPECT, is not abnormal in GTS.

Key Words. Single photon emission computed tomography, neuroleptics, ^{123}iodo-6-methoxybenzamide, tics.

Gilles de la Tourette Syndrome (GTS) is a movement disorder characterized by chronic motor and vocal tics (Robertson, 1989). The anatomical location, frequency, complexity, and severity of the tics change over time, and the onset of the disorder characteristically occurs before the age of 14 years (American Psychiatric Association, 1987; Bruun, 1988). Although the exact prevalence of GTS is unknown, a currently accepted figure is 0.5 per thousand (approximately 110,000 patients in the United States and 27,500 in the United Kingdom) (Bruun, 1988), but even this may prove to be an underestimate. GTS is found worldwide and in all cultures, racial

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groups, and social classes (Robertson, 1989). It occurs three or four times more commonly in males than in females.

The etiology of GTS is now generally thought of as being biological, rather than psychodynamic, although stress exacerbates tics. Some authors have suggested an autosomal dominant gene involving tics, GTS, and obsessive-compulsive disorder (OCD) (Pauls et al., 1986; Robertson, 1989). The neurochemical basis for GTS is, as yet, not known, with several putative neurotransmitter abnormalities already reported (Caine et al., 1988; Singer et al., 1990). However, the neurotransmitter dopamine has received the most attention, mainly because dopamine-blocking agents (e.g., haloperidol) reduce GTS symptoms, while dopaminergic stimulants (e.g., pemoline, methylphenidate) worsen tics and related behavior. There are many potential steps at which dopamine dysfunction might occur in GTS (e.g., overproduction, decreased catabolism, abnormal receptor binding, heightened post-receptor signal) (Singer et al., 1991). Genetic studies, however, have failed to find linkage to the D_1 or D_2 receptor (Gelernter et al., 1990).

To investigate the possibility of abnormal D_2 receptor binding or availability, we used brain-dedicated multidetector single photon emission computed tomography (SPECT) (George et al., 1991) and the recently developed D_2/D_3-receptor ligand, ^{123}iodo-6-methoxybenzamide (^{123}I-IBZM) (Kung et al., 1989, 1990) to examine D_2/D_3-receptor activity in GTS patients and control subjects.

Methods

Subjects. GTS subjects were recruited from the Gilles de la Tourette Syndrome Clinic at The National Hospitals for Neurology and Neurosurgery, Queen Square, London, U.K. The referral pattern and demographic makeup of the GTS clinic has been previously described (Robertson et al., 1988; Robertson, 1989). Approximately one to two new GTS patients are evaluated per week from throughout the U.K., with a referral base of more than 56 million. Subjects were initially examined by a neuropsychiatrist (M.M.R.) to ascertain the diagnosis of GTS according to *DSM-III-R* criteria (Table 1) and then confirmed by another neurologist/psychiatrist (M.S.G.) before study entry. Additional measures included the National Hospital Tourette Interview and a specially chosen battery of standardized psychiatric rating scales to measure specific aspects of psychopathology (Robertson et al., 1988; Robertson, 1989). The mean duration of illness was 10 years (SD = 9.8), with a mean tic severity of 65 on the Yale Global Tic Severity Scale (Leckman et al., 1989). Exclusionary criteria for both patients and volunteers included hepatic, renal or cardiac disease, pregnancy, seizure disorder, mental retardation, past history of cocaine or stimulant abuse, encephalitis, exposure to methylphenidate, or the presence of depression or severe psychiatric illnesses (other than OCD or GTS in the case of the patients).

Healthy volunteers were recruited from hospital staff at the Institute of Psychiatry using the inclusion and exclusionary criteria defined above. This research was approved by the ethics committees of The National Hospitals for Nervous Diseases, The Middlesex Hospital, and ARSAC (the U.K. regional radiation safety committee). Informed written consent was obtained from each individual.

Procedures. Scanning was done using a brain-dedicated single-slice tomograph, the Strichman Medical Equipment (SME) 810 Brain Tomograph, at the Institute of Nuclear Medicine (UCMSM). Subjects were placed supine on the SME 810 couch. The orbitomeatal line (OM line) was visually aligned with the gantry to obtain OM parallel slices. Dynamic

Table 1. Subject data

Subject #	Age (years)	Sex	Medication (time since last taken)	OCD	ADHD	Handedness
1	27	F	Haloperidol, 3 months	+	—	R
2	45	F	Sulpiride, 4 months	+	—	R
3	29	M	Never medicated	+	—	R
4	10	M	Never medicated	—	—	R
5	15	M	Sulpiride, 3 months	—	—	R
6	31	F	Never medicated	+	—	R
7	12	M	Never medicated	+	—	R
8	15	M	Pimozide, 3 months	+	—	R
Medicated mean						
	23	5/3	(M/F)	6/2	0/8	
9	47	M	Clomipramine, 100 mg/day Sulpiride, 800 mg/day	+	—	R
10	27	M	Clomipramine, 100 mg/day Sulpiride, 80 mg/day	—	+	R
11	14	F	Haloperidol, 5 mg/day	—	—	R
12	38	M	Flupenthixol, 40 mg/4 wks	—	+	R
13	12	M	Sulpiride, 200 mg/day	+	+	R
14	16	M	Flupenthixol, 40 mg/2 wks	—	—	R
15	14	M	Sulpiride, 400 mg/day Clomipramine, 30 mg/day	+	+	R
Medicated mean						
	24	6/1	(M/F)	3/4	4/3	
Control mean						
	30	4/2	(M/F)	0	0	All R

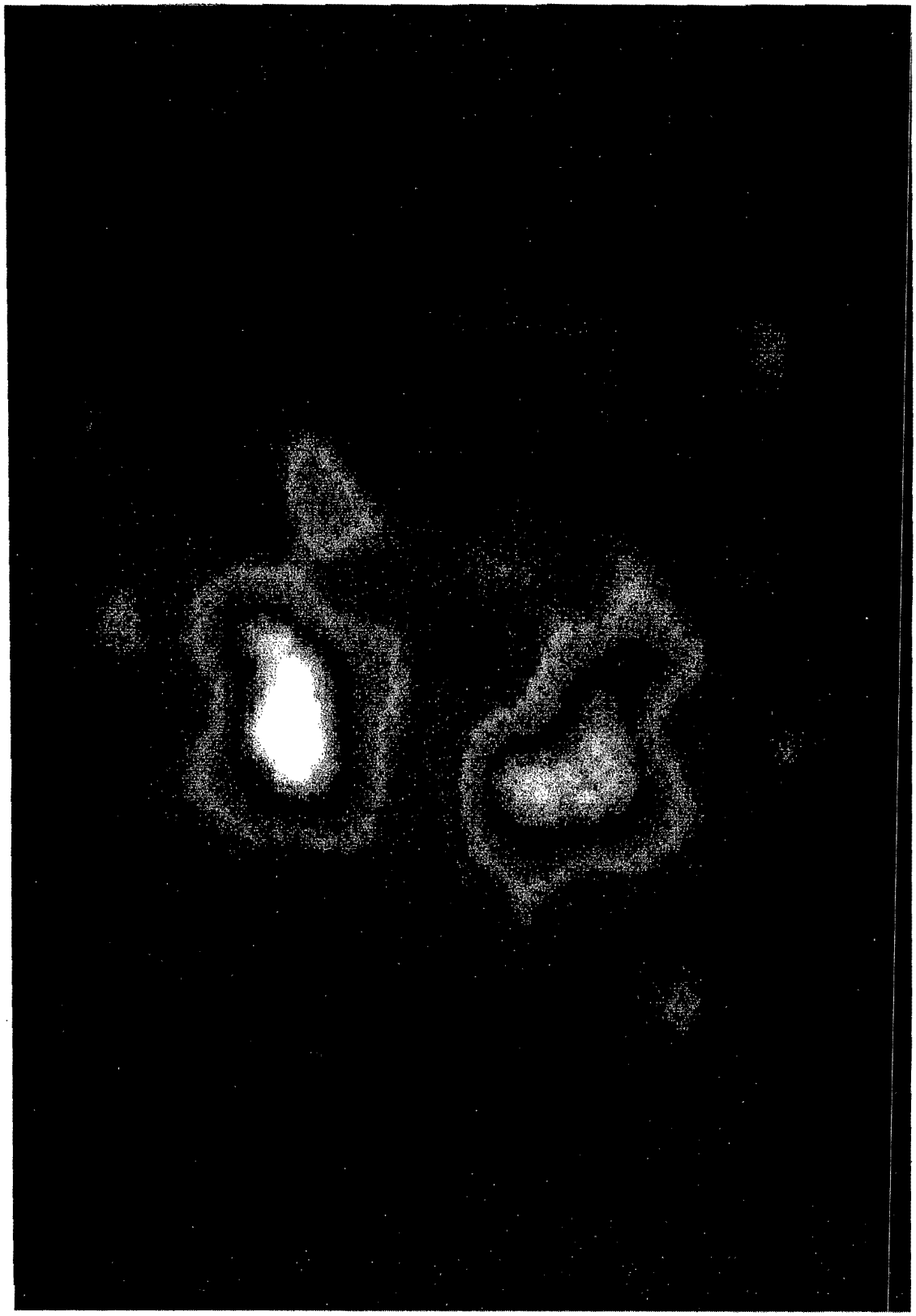
Note. OCD = obsessive-compulsive disorder. ADHD = attention deficit hyperactivity disorder. F = female. M = male. R = right. L = left.

keep their eyes open and not to talk. After resting quietly for 1-2 minutes, subjects were given an intravenous injection of ^{123}I-IBZM through a 19-gauge needle in the right antecubital vein. The average dose of 5 mCi (185 MBq) was flushed through with 20 ml of saline. (^{123}I-IBZM was supplied by Cygne B.V., University of Eindhoven, The Netherlands.) Binding of ^{123}I-IBZM peaks 30-40 minutes after the injection and has a linear washout rate, resulting in a relatively stable target-to-background ratio of activity for up to 2 hours after the injection (Kung et al., 1989, 1990; Costa et al., 1990; Brucke et al., 1991).

Scanning began immediately after the injection and continued for as long as the subjects could accept. Most subjects and volunteers tolerated at least 55 minutes of scanning, and thus the data analysis for this study is limited to 55 minutes. Acquisition time per slice was 5 minutes with a 1.5-minute interscan interval. The in-plane resolution of this system is 7-9 mm (full width at half maximum). The average slice thickness was 1.25 cm. Images were then reconstructed using SME software on an Apple MacIntosh computer.

Image Analysis. Regions of interest (ROIs) were drawn with a mouse around the entire frontal lobe, entire visual cortex, and the right and left basal ganglia by a neurologist (M.S.G.) who was unaware of diagnosis at the time of the analysis. A template for the right and left basal ganglia was drawn around the 60-minute image with the 70% isocolor contour (Fig. 1). Frontal lobe and visual cortex templates were drawn on the 10-minute image (blood flow/perfusion dependent distribution of ^{123}I-IBZM) at the 40% isocolor contour. The same

Fig. 1. Transverse ^{123}I-IBZM image at 60 minutes after injection with the color scale adjusted such that the 70% isocolor contour defines peak basal ganglia definition



^{123}I-IBZM = ^{123}iodo-6-methoxybenzamide. The 60-minute image was used for optimal placement of the basal ganglia region of interest used for all subjects.

templates were used for all scans, adjusted to fit optimally over the defined regions. This ROI sampling method has a 2.2% mean variation in the regions used.

Statistical Analysis.

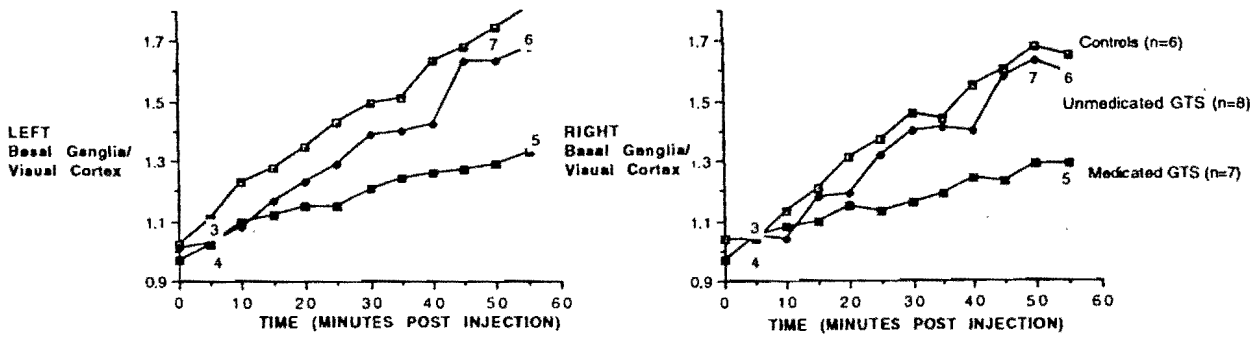
Ratios. Because numerous factors can affect the total count rate in a given patient (e.g., amount of ligand absorbed, ligand remaining in the syringe), basal ganglia to visual cortex (BG/VC) and basal ganglia to frontal cortex (BG/FC) ratios were used to express relative ^{123}I-IBZM regional activity and produce a qualitative index of D_2 receptor availability. The regions used included both hemispheres.

Similar results emerged when basal ganglia to frontal cortex (BG/FC) ratios were used. However, some of the GTS patients in this study also participated in a SPECT blood flow study that found increased right frontal blood flow in GTS subjects compared with control subjects (George et al., 1992). Thus, to avoid possible confounds due to changes in frontal blood flow, we present only BG/VC ratios.

Relative binding curves. Mean BG/VC ratios for the left and right hemispheres were computed by group (control subjects, unmedicated and medicated GTS patients) for each time data point (from 0 to 55 minutes after injection). To produce a relative binding curve for ^{123}I-IBZM, group right and left BG/VC ratios were expressed over time (Fig. 2). Group means of each BG/VC value at each time point were compared by two-way repeated measures analysis of variance (ANOVA) (3 groups × time) for each hemisphere, with post hoc Student-Newman-Keuls tests for significance.

Slopes of binding curves. The ANOVA cannot deal with missing data points, and thus 9/21 cases were rejected. These initial and late missing data points were due to patient movement, artifact, or the inability to tolerate 55 minutes of scanning (see Fig. 2). To test whether the results were erroneously influenced by the small number of subjects that entered the ANOVA, we used an additional method of data analysis. Each subject's relative binding curve for each hemisphere from 0 to 55 minutes was plotted on a Macintosh computer with commercial software (Cricket Graph) which then computed a simple linear (first-degree polynomial) equation ($y = mx + b$) to fit each subject's relative binding curve. Initially, we performed a hemisphere × group ANOVA to assess hemispheric differences. Then, for both the right and left hemispheres, the mean slopes for each group were compared by one-way ANOVA, with post hoc *t* tests (Student-Newman-Keuls procedure).

Fig. 2. ^{123}I-IBZM binding curves of the right and left basal ganglia/visual cortex over time by groups (medicated GTS patients, unmedicated GTS patients, and control subjects)



^{123}I-IBZM = ^{123}iodo-6-methoxybenzamide. GTS = Gilles de la Tourette's Syndrome. For both the right and left basal ganglia, the medicated GTS group shows differences that became apparent at 20 minutes after injection. The unmedicated group did not differ significantly from control subjects. The numbers immediately above a graphed point indicate where the number of subjects included in the group mean is less than the total stated on the right due to motion or artifact.

Results

Two-Way Repeated Measures ANOVA (3 Groups × Time).

Within subjects (time effect). For both hemispheres, a significant time effect occurred, demonstrating significant binding of the ligand to receptors over time ($n = 13$; right: $F = 27.72$; $df = 9, 90$; $p < 0.001$; left: $F = 38.89$; $df = 9, 90$; $p < 0.001$).

Between subjects (group effect). A significant between-subject group effect was found in the right basal ganglia ($F = 6.07$; $df = 2, 10$; $p = 0.019$). This was due to a significant difference between normal control subjects and medicated GTS subjects ($n = 13$ total; 6 control subjects: mean BG/VC slope = 1.31, SD = 0.11; 4 medicated GTS patients: mean BG/VC slope = 1.12, SD = 0.03; 3 unmedicated GTS subjects: mean BG/VC slope = 1.19, SD = 0.09; $p < 0.05$).

A significant between-subjects group effect was found. In the left basal ganglia ($F = 8.08$; $df = 2, 10$; $p = 0.008$). Post hoc tests revealed that this was due to significant differences between medicated GTS patients and control subjects, and between unmedicated GTS patients and control subjects ($n = 13$ total; 6 control subjects: mean BG/VC slope = 1.37, SD = 0.10; 4 medicated GTS patients: mean BG/VC slope = 1.18, SD = 0.025; 3 unmedicated GTS patients: mean BG/VC slope = 1.24, SD = 0.15; $p < 0.05$).

One-Way ANOVA of Uptake Slopes × Group. A group × hemisphere ANOVA revealed significant effects of group ($F = 8.91$; $df = 2, 18$; $p = 0.002$) and hemisphere ($F = 5.95$; $df = 1, 18$; $p = 0.025$), although there was no significant group × hemisphere interaction. For both the right and the left basal ganglia, the medicated group had significantly lower slopes (reflecting lower D_2/D_3 binding over time) than control subjects or unmedicated GTS patients on the basis of post hoc Student-Newman-Keuls tests. Other differences (i.e., between unmedicated GTS patients and control subjects) were not significant.

A significant between-group difference existed for the right and left BG/VC mean slopes. On the right, this difference ($F = 5.86$; $df = 2, 18$; $p = 0.01$) was attributable to a significantly lower group mean slope ($p < 0.05$) in medicated GTS patients ($n = 7$; mean group slope = 0.001943, SD = 0.0048) than in control subjects ($n = 6$, mean group slope = 0.0064, SD = 0.0022), and between medicated GTS patients and unmedicated GTS patients ($n = 8$, mean slope = 0.008388, SD = 0.0034). On the left, this significant difference ($F = 10.45$; $df = 2, 18$; $p = 0.001$) was attributable, as it was on the right, to significant differences ($p < 0.05$) between medicated GTS patients ($n = 7$, mean slope = 0.003129, SD = 0.003087) and control subjects ($n = 6$, mean slope = 0.0072, SD = 0.002158) and between medicated GTS patients and unmedicated GTS patients ($n = 8$, mean slope = 0.010450, SD = 0.003625).

More consistent results were found with the slopes method, which used a larger number of subjects, than with the repeated measures ANOVA.

Discussion

Dopamine Theory. There are several reasons for believing that dopamine may be involved in the pathophysiology of GTS. The main argument for dopamine's

involvement in GTS comes from observations of treatment effects. The brain dopamine neurotransmitter system acts through at least two different receptors, D_1 and D_2 (Singer et al., 1991), although more are being identified and cloned. The affinities of neuroleptics for D_2 sites are closely correlated with the amelioration of psychotic and movement disorders (Singer et al., 1991). Thus, the D_2 receptor could play a crucial role in GTS since the largest class of medications that successfully treat GTS are D_2 receptor antagonists such as haloperidol (Shapiro and Shapiro, 1982), pimozide (Golden, 1984; Colvin and Tankanow, 1985), and sulpiride (Robertson et al., 1990). Some GTS patients, however, do not benefit from neuroleptic medication (Robertson, 1989). It is therefore possible that responsiveness to dopamine (D_2) antagonists may identify two distinct subgroups of GTS patients.

Another argument for the involvement of dopamine in GTS comes from the fact that dopaminergic agonists such as L-dopa (Sacks, 1982) and central nervous system (CNS) stimulants such as methylphenidate (Golden, 1984) have an adverse effect on GTS symptoms, while dopamine depletors such as tetrabenazine (Jankovic et al., 1984) are associated with moderate improvement. The monoamine metabolite homovanillic acid has been found to be decreased in the cerebrospinal fluid of some GTS patients, although the methods involved have been criticized (Caine, 1985).

Neuroimaging in GTS. Several groups are currently using imaging techniques to investigate abnormalities in GTS, and their results suggest that the abnormalities involve both CNS function and structure. Robertson et al. (1988) reported 71 out of 73 patients with GTS to have normal findings on computed tomography (CT). Other studies have noted CT scan abnormalities in 16 GTS patients; only 18 out of 172 documented CT scans have been abnormal, and the abnormalities do not appear to be of direct etiological significance (Robertson, 1989). Recently, two separate studies have found reduced basal ganglia volumes in GTS patients compared with control subjects. Peterson et al. (1993) studied 14 adult GTS patients and found them to have decreased volume, as indicated by magnetic resonance imaging, in the left lenticular nuclei compared with findings in age-matched control subjects. In another MRI study, Singer et al. (1993) found that 37 GTS children had decreased volume in their left globus pallidus compared with their right, an asymmetry that was not present in 18 control subjects.

An initial study with positron emission tomography (PET) that used ^{18}F-deoxyglucose found abnormalities in five GTS patients compared with control subjects (Chase et al., 1984). GTS patients showed a relatively close positive association between metabolism in the basal ganglia (particularly the corpus striatum) and metabolism throughout the cerebral cortex. Vocal tic severity varied inversely with glucose metabolism in the middle and inferior parts of the frontal lobes bilaterally, extending posteriorly from the frontal poles to the postcentral gyrus. Coprolalia, in contrast, was inversely correlated with hypometabolism in the left parasylvian region (Chase et al., 1984). In a more recent study that used a PET scanner with higher resolution and sensitivity, Chase et al. (1986) compared 12 untreated GTS patients with matched normal control subjects. At horizontal levels from 8.4-8.8 cm caudal to the vertex, nonnormalized glucose utilization rates were approximately 15% below control values in the region of the frontal cingulate and possibly insular cortex and in

the inferior corpus striatum ($p < 0.01$). Further studies by the same group (Braun et al., 1991; Stoetter et al., 1991) have revealed focal neuroanatomical abnormalities that primarily involve portions of the putamen and frontal and limbic cortices. In a recent SPECT study with technetium-99m-d,l-hexamethyl-propylene amine oxime as tracer, George et al. (1992) found elevated frontal cerebral blood flow in 20 unmedicated GTS patients compared with eight healthy volunteers. Finally, Singer et al. (1992) failed to find differences in dopamine binding between GTS patients and control subjects in a PET study with ^{11}C-*N*-methylspiperone as the ligand.

Obsessive-compulsive disorder (OCD) is believed by some investigators to be genetically, phenomenologically, and probably neuroanatomically linked to GTS (Pauls et al., 1986; Baxter, 1990; George, 1992). Studies of brain structure in OCD are conflicting, with some reporting decreased caudate volume in OCD (Luxembourg et al., 1988) and others not (Garber et al., 1989; Kellner et al., 1991). Functional neuroimaging studies of OCD using ^{18}F-deoxyglucose PET have consistently revealed increased glucose metabolism in the caudate nuclei and orbital frontal lobes (Nordahl et al., 1989; Swedo et al., 1989; Baxter, 1990).

Interpretation of Results. As would be predicted, GTS patients taking dopamine-blocking medications displayed decreased ^{123}I-IBZM activity in both basal ganglia, consistent with blocked D_2/D_3 receptors. Activity was significantly lower than that in healthy control subjects and unmedicated GTS patients. Two separate methods of data analysis corroborate this finding.

In addition, this study failed to demonstrate significant differences between unmedicated GTS patients and control subjects in basal ganglia D_2/D_3 uptake. The repeated measures ANOVA did find a marginally significant difference on the left, but this analysis used only three subjects and the effect was not significant when the the mean slope method was used.

Some caution should be used in interpreting these results. The numbers were small (eight unmedicated GTS patients, seven medicated GTS patients, and six control subjects). Thus, a Type II error cannot be excluded. We considered and excluded the possibility of "contamination" or mislabeling of a patient taking occult D_2 blocking agents as being unmedicated. The unmedicated group consisted of four drug-naive subjects and four subjects who had not taken medications for more than 3 months. Subanalyses of these two subgroups revealed similar results.

Differences in regional brain volume could conceivably have influenced our results. The most recent CT and MRI evidence indicates that GTS subjects have abnormally small left basal ganglia or globus pallidus. This decrease in left-sided caudate volume might explain the transient lag in GTS caudate D_2 receptor binding (Fig. 2). Thus, decreased caudate volume, with decreased initial first pass of IBZM, might influence or partially explain our present results. We are investigating subjects in this study with MRI to quantitate volume of the basal ganglia, frontal cortex, and visual cortex.

Finally, there are methodological questions about the specific ability of ^{123}I-IBZM to identify D_2/D_3 receptors and the precise time at which the SPECT picture changes from one of general blood flow to more specific D_2 binding. Studies show that ^{123}I-IBZM binding to postsynaptic D_2 receptors begins reliably at 20 minutes after injection, with peak binding at 35-40 minutes after injection (Kung et al., 1989, 1990; Costa et al., 1990; Brucke et al., 1991; Malison et al., 1991). These results could also be influenced by different levels of endogenous dopamine between control subjects and GTS subjects. For example, it is conceivable that GTS subjects might have higher levels of D_2/D_3 receptors, but that the higher number is offset by higher resting levels of endogenous dopamine, thereby occupying and blocking some of the receptors.

The present findings do not appear to support the theory of heightened or excessive D_2 receptor binding of dopamine in GTS. The preliminary findings need to be replicated in other centers and with other D_2 ligands, however, before firm conclusions can be drawn. It is to be hoped that functional neuroimaging studies with specific neuroligands will ultimately prove to be a powerful tool in unraveling the pathophysiology of Tourette's Syndrome.

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Dopamine transporter binding in Gilles de la Tourette syndrome: a [^{123}I]FP-CIT/SPECT study

Serra-Mestres J, Ring HA, Costa DC, Gacinovic S, Walker Z, Lees AJ, Robertson MM, Trimble MR. Dopamine transporter binding in Gilles de la Tourette syndrome: a [^{123}I]FP-CIT/SPECT study.
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Objective: To investigate dopamine transporter binding in Gilles de la Tourette syndrome (GTS) with SPECT and [^{123}I]FP-CIT.
Method: Ten neuroleptic naïve/free patients with GTS, and 10 age- and gender-matched normal volunteers were studied. Subjects were clinically evaluated. GTS severity and affective symptoms were measured and the presence of GTS-related behaviours were recorded.
Results: The GTS group showed significantly higher binding in both caudate and putamen nuclei than the controls. No associations were found between striatal binding ratios and measures of affect or GTS-related behaviours.
Conclusion: Patients with GTS show higher striatal binding of FP-CIT to the striatum in comparison with age- and gender-matched control subjects, indicating that dopamine transporter abnormalities are involved in the pathophysiology of GTS. These abnormalities appear to be distributed across both caudate and putamen.

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Key words: dopamine transporters; Tourette's disorder; SPECT

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Introduction

Gilles de la Tourette syndrome (GTS), also known as Tourette's disorder, is a neuropsychiatric condition characterized by the presence of multiple motor and one or more vocal (phonic) tics, with an onset before the age of 18 years (1). Its prevalence has been suggested to be of the order of five per 10 000 (2). In addition, some patients may present with coprophenomena, echophenomena, palilalia and a variety of other neurobehavioural disorders such as obsessive–compulsive phenomena, depression, self-injurious behaviour and attention deficit hyperactivity disorder (2).

The biological basis of GTS is not known yet and functional brain imaging studies using PET and SPECT have reported various findings in GTS patients. Considering markers of regional cerebral activity, findings of both increase and decrease in activity have been noted. Braun et al. (3) reported decreases in glucose metabolism in midbrain and ventral striatum and Eidelberg et al. (4) described metabolic decreases in caudate and thalamus. Moriarty et al. (5) similarly noted hypoperfusion of the left caudate, the anterior cingulate cortex and the left dorsolateral prefrontal cortex, as well as found that tic severity correlated with hypoperfusion in the left caudate, the cingulate and left medial temporal regions. However, George et al. (6) observed elevated frontal cerebral flow in their group of patients with GTS.

Although the neurochemical basis of GTS is unclear, there is evidence of involvement of the dopaminergic system, based on the observations of the beneficial effects of dopamine blocking agents on the motor symptoms of GTS and of the exacerbation of these symptoms with dopamine enhancing drugs such as methylphenidate (2, 7) and cocaine (8). In addition, homovanillic acid, the metabolite of dopamine, has been reported to be decreased in the cerebrospinal fluid of patients with GTS (2).

Several aspects of dopaminergic system activity have also been investigated in patients with GTS.

ee neuroimaging studies of presynaptic (using T and fluoro-dopa) and postsynaptic (D2) pamine receptors (using PET and [C^{11}]raclode and SPECT and [^{123}I]IBZM) have failed to nonstrate any abnormalities in dopaminergic eptor systems (9–12). However, Wolf et al. (13) orted greater IBZM binding to striatal D2 eptors in the clinically more affected sibling of n pairs in their SPECT study.

n contrast with these findings, two PET studies dopamine re-uptake sites and DOPA decarbylase activity using [C^{11}]WIN 35 428 and [^{18}F]-OPA, respectively and more recent ones using ECT and [^{123}I]ß-CIT, have demonstrated reased binding to presynaptic striatal dopamine uptake sites in GTS patients when compared th control subjects (14–16) and higher striatal OPA ratios (17). These results are also in eping with a postmortem study of striatal pamine re-uptake sites in patients with GTS ing [^{3}H] mazindol, in which an increased binding as observed in patients with respect to control bjects (18). It was speculated that these findings uld be explained by an enhanced dopaminergic nervation within the striatum. In contrast, two her [^{123}I]ß-CIT/SPECT studies found no differnces between GTS patients and normal control bjects in binding ratios to striatal dopamine ansporter molecules (19, 20). A recent study sing PET and DTBZ, to measure binding to riatal vesicular monoamine transporter type-2 VMAT2), found no evidence of increased binding n GTS patients (21).

im of the study

he aim of the present study was to further nvestigate the findings of previous studies of opamine transporter binding in patients with GTS by using high resolution SPECT and [^{123}I]FP-CIT in a sample of GTS patients and ge- and gender-matched normal volunteers.

Material and methods

Subjects and assessments

The project received ethical approval by the National Hospital for Neurology and Neurosurgery and the Institute of Neurology (UCL) and also by the Administration of Radioactive Substances Advisory Committee (ARSAC). Written consent was obtained from all the subjects. Ten consecutive patients with GTS meeting DSM-IV criteria (1) were studied. Eight patients were recruited from the GTS clinic at the National Hospital for Neurology and Neurosurgery, London. The referral pattern and demographical makeup of this clinic has been previously described (22, 23). The patients were initially examined by a neuropsychiatrist (MMR) to ascertain the diagnosis of GTS and re-assessed by another neuropsychiatrist (JS-M) before study entry to establish symptoms near the time of scanning. Two GTS patients were recruited via the UK Tourette's Association and were assessed by a neuropsychiatrist (JS-M) prior to being studied. They had both been previously diagnosed as suffering from GTS by consultant neurologists elsewhere.

Exclusion criteria were: drug and/or alcohol dependence, abuse of cocaine, ecstasy or amphetamines and derivatives, current treatment with methylphenidate, current or recent treatment with dopamine stores depleting agents (such as tetrabenazine), current psychotic or other severe psychiatric illness (other than OCD), presence of general medical conditions including neurological disorders other than GTS, history of head injury, pregnancy, learning disability, treatment with neuroleptic drugs within 2 months preceding the scan and age outside the range of 18–70 years.

Information was obtained on duration of the disease and current and past medications. Severity of GTS was measured with the Yale Global Tic Severity Rating Scale (24), which gives total scores on motor symptoms, vocal tics, general impairment and a global score. Presence of current Attention Deficit Hyperactivity Disorder (ADHD) was checked by applying DSM-IV (1) criteria. Affective and anxiety symptoms were measured with the Hospital Anxiety and Depression Scale (HAD) (25). Obsessive-compulsive phenomena were assessed with the Leyton Obsessional Inventory (LOI) (26). The presence or absence of self-injurious behaviour (SIB), echophenomena and coprophenomena during the course of the disorder were recorded.

All but one of the patients were 'neuroleptic-naïve' and this subject had not taken any antipsychotic drug for more than 1 year. None of the patients had taken any selective serotonin re-uptake inhibitor for 2 months prior to the study and none of them had ever taken clonidine.

The GTS patients were compared with 10 age- and gender-matched healthy volunteers. Patients were matched by age with control subjects within a range of 10 years, because striatal binding to dopamine transporter molecules declines between 6.6 and 10% per decade in normal subjects (27, 28). The same exclusion criteria applied to control subjects as to the GTS group. No control subject achieved caseness in any of the assessments

described above. The subjects recruited were from among the hospital staff, staff from a hostel for the homeless, and spouses of patients with Parkinson's disease and dementia with Lewy bodies, and underwent a clinical interview with a neuropsychiatrist (JS-M).

Radiopharmaceutical and scanning protocol

The radiopharmaceutical [^{123}I]FP-CIT or 2ß-carbomethoxy-3ß-(4-iodophenyl)-*N*-(3-fluoropropyl) nortropane has been developed by Neumeyer et al. (29) for the imaging of the dopamine transporter (DAT) in the living human brain. It is a tropane analogue and hence a cocaine congener. Pseudo-equilibrium of this tracer in the basal ganglia occurs between approximately 3–6 h postinjection (30).

[^{123}I]FP-CIT was supplied by Nycomed Amersham plc. (Little Chalfont, Bucks, UK). All subjects received potassium iodide 120 mg/day orally in a single dose for 2 days prior to the scan, on the day of the scan and for 2 days after the scan, to block thyroid uptake of ^{123}I. On arrival for the scanning session, subjects were asked to sit quietly on a chair and a 19-gauge cannula was inserted in the right antecubital vein. Between 10 and 15 min later the radiotracer was injected over a period of 20 s, followed by a 20 ml flush of normal isotonic saline solution. Each subject received approximately 185 MBq of [^{123}I]FP-CIT.

Data acquisition

Data acquisition started between 3.5 and 5 h postinjection during pseudo-equilibrium (basal ganglia over background radioactivity ratios are almost constant between 3 and 6 h postinjection). Acquisition time was between 30 and 35 min (5 min per slice). The scans were performed in a brain-dedicated single-slice tomographic scanner (SME 810 Brain Tomograph: Strichman Medical Equipment, Medfield, MA, USA) at the Institute of Nuclear Medicine (University College London Medical School). The SME is made of 12 detectors equipped with high resolution (800 holes) collimators, arranged on a circle around the patient's head. The number of counts acquired per slice at the basal ganglia level was approximately 500 000. The reconstructed spatial resolution measured using the high resolution collimators at our institution was 8.1 mm at the centre and 8.7 mm full-width half maximum (31). Subjects were placed supine on the scanner's couch. The orbitomeatal line was visually aligned with the gantry to obtain orbitomeatal parallel slices. At least three contiguous slices were acquired from 25 to 55 mm above the orbitomeatal line. The subjects' heads were strapped to the head rest with tape around the forehead and the chin. Subjects were instructed to lie quiet and not to talk. No significant head movement was observed during scanning. Data acquisition followed manufacturer's recommendations.

Data processing

Data processing was carried out by DCC following manufacturer's recommendations. Iterative reconstruction was employed and Hanning-type filters were used to obtain good contrast resolution with attenuation correction. Hanning filters are set by the manufacturers and never changed.

Data analysis

Circular regions of interest (ROI), all of the same size (111 pixels), were used to calculate striatum/occipital radioactivity ratios for the caudate nucleus and putamen (anterior or rostral and posterior or caudal) on each hemisphere. Placement of the ROI was always made by the same observer (DCC), according to the anatomical visualization and tracer distribution, and following Tailarach coordinates. For the striatum, one ROI for the caudate nucleus, one for the anterior putamen and another for the posterior putamen were placed with the pixel with maximum counts on the centre of each ROI. In the occipital cortex, five ROI on each hemisphere were displayed along the calcarine cortex and the accessory visual cortex. Average counts per pixel were obtained from each striatal region on three contiguous slices with the highest radioactivity signal. Ratios were calculated with the average counts per pixel on the three striatal slices and ten ROI per slice placed in the visual cortex. The latter were used as the denominator. Simple radioactivity ratios were then calculated between each of the striatal ROI and the occipital cortex.

Results

Clinical results

There were six males and four females in each study group. Data for the emotional state of one control subject were accidentally lost. The mean age in the GTS group was 43.5 years (range = 18–64, SD = 13.15) and in the control group 45.7 years (range = 28–67, SD = 14.76). The mean ages in the two groups were not significantly different (1-way ANOVA, $F_{(1,18)} = 0.124$, $P = 0.7$).

Table 1 shows the clinical characteristics of the GTS group. In this group there were two subjects with echolalia, one with echopraxia and two with both. Two subjects had coprolalia, two had copropraxia and one had both phenomena. There were six subjects with SIB and five subjects had childhood ADHD, but not the current disorder (DSM-IV) (1).

The two study groups were significantly different in measures of depression and anxiety, and obsessive-compulsive symptoms; with GTS patients scoring higher than controls in all measures (Table 2).

SPECT results

The GTS group as a whole showed a higher level of binding in caudate and putamen nuclei compared with the control group, and this difference was statistically significant (1-way ANOVA) (Table 3). To account for the fact that variances in striatal binding ratios in the GTS group may not be normally distributed, binding ratios for the two groups were also compared using non-parametric tests (Kruskal–Wallis Test). This showed that differences in binding ratios remained significant (all ratios $P < 0.05$).

Considering the GTS group, there were no significant correlations (bivariate) between total tic severity and binding to the basal ganglia sites which were studied. Similarly, within the GTS group there were no significant correlations between basal ganglia binding and depression or anxiety scores, obsessive–compulsive symptoms, or childhood ADHD; nor were there any significant differences in binding between those GTS patients with echophenomena, coprophenomena or SIB and those without such symptoms (1-way ANOVA). No associations were found between striatal binding ratios and age within either of the study groups (bivariate correlations). There were significant bivariate correlations between disease duration and binding to right caudate ($r = -0.65$, $P = 0.04$), left caudate ($r = -0.66$, $P = 0.039$) and right posterior putamen ($r = -0.66$, $P = 0.036$), indicating that the shorter the duration, the higher the binding. Closer inspection of regional binding data from the individual subjects revealed that those subjects with higher DAT binding tended to be younger and to have a shorter duration of their disease. Further analysis was not conducted because of the small sample sizes.

Table 1. Clinical characteristics of the GTS group

	Mean	Range	SD
Age	43.50	18–64	13.15
Duration	35.00	13–57	14.66
YGTSS (motor)	12.50	9–21	3.75
YGTSS (phonic)	8.10	2–18	4.36
YGTSS (total)	47.60	27–68	13.27

YGTSS = Yale Global Tic Severity Scale.

Table 2. Mean scores on psychopathological scales for both groups

	GTS*			Controls†		
	Mean	Range	SD	Mean	Range	SD
HAD (dep.)	8.80	0–18	6.84	2.00	0–5	1.60
HAD (anx.)	10.90	5–21	6.03	2.75	1–6	1.67
LOI (total)	34.20	15–53	14.23	11.56	5–26	6.33

HAD = Hospital Anxiety and Depression Scale, LOI = Leyton Obsessional Inventory.
* GTS ($n = 10$).
† Controls ($n = 9$).

Table 3. Mean binding ratios in the two study groups

	GTS		Control	
	Mean	SD	Mean	SD
LAP*†	6.58	1.26	5.51	0.72
LCN*†	7.40	1.37	6.07	0.72
LPP*†	5.43	0.89	4.70	0.55
RAP*†	6.80	1.34	5.65	0.65
RCN*†	7.22	1.15	6.22	0.78
RPP**†	5.94	1.16	4.71	0.56

RCN = right caudate, LCN = left caudate, RAP= right anterior putamen, RPP= right posterior putamen, LAP = left anterior putamen, LPP = left posterior putamen.
GTS = Gilles de la Tourette syndrome, SD = standard deviation.
* $P < 0.05$ (1-way ANOVA).
** $P < 0.01$ (1-way ANOVA).
† all $P < 0.05$ (Kruskal–Wallis).

Discussion

In this study, it was found that patients with GTS show significantly higher binding of FP-CIT to striatal DAT molecules than age- and gender-matched healthy volunteers. The high resolution methods used in this study allowed the differentiation between the various components of the striatum: caudate nucleus and anterior and posterior aspects of the putamen. This is an advantage over the previous *in vivo* studies that have, at most, separated caudate from putamen (11, 14–20). This study, as far as known, is the first one to define three ROI in the striatum corresponding with the caudate nucleus and the two divisions of the putamen nucleus, and also the first study using FP-CIT in GTS. It is therefore noteworthy that increased binding ratios were observed in each of these different regions of the basal ganglia.

Measures of psychopathology frequently associated with GTS, such as obsessive–compulsive behaviours, depressive symptomatology, or anxiety, did not show any associations with striatal binding to DAT. The same was true for echo and coprophenomena and SIB. This may suggest that these behavioural abnormalities, whilst related to some aspect of the pathophysiology of GTS, do not arise out of disturbances in striatal DAT binding.

Higher striatal binding to DAT in GTS patients has already been observed in previous *in vivo* (16, 17) and postmortem studies (18). The significance of this finding is still a matter of speculation, especially in relation to whether it is a primary or a secondary phenomenon. An increase in binding to DATs could be explained by a hyperinnervation of the dopaminergic system (18) leading to increased number of re-uptake sites, or by an increase in the concentration of re-uptake sites on normal numbers of dopaminergic terminals (18).

Hyperinnervation should also cause an increase in the release of dopamine, but as Singer et al. (18) have suggested, this would be incompatible with the reported finding of low levels of homovanillic acid (HVA) in CSF of GTS patients. They speculated that low HVA is because of an enhanced clearance of dopamine from the synapse by increased numbers of DATs (18). Singer et al.'s study (18) has also shown normal levels of tissue dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and HVA. An increased dopaminergic innervation could explain why D2 receptor blockers or dopamine storage blockers improve tics (18).

Low levels of endogenous synaptic dopamine should cause an increased binding to postsynaptic D2 receptors. However, till date, only one study has found increased IBZM binding to D2 receptors (in monozygotic twins discordant for GTS) (13), while others have not observed any abnormalities (10–12, 18, 32).

Both postmortem and structural neuroimaging studies in GTS have reported morphometric abnormalities in components of the striatum. In the former, it has been observed that in these structures there are greater number of neurones but of a smaller size (33) and a greater number of dopamine transporters (18). In the latter, smaller striatal volumes have been observed (34–36). Reduced striatal volumes may have contributed to the reduced striatal blood perfusion and glucose metabolism in GTS reported by some authors (3, 5, 37).

In this study, a shorter duration of the symptomatology tended to be associated with higher bilateral caudate and right posterior putamen binding to DATs, but as there was no correlation between age and binding, this finding is not explained simply as an age effect. However, it may relate to the observations that peak tic severity occurs during adolescence and then gradually decreases over time (38).

Possible confounding factors in this study will now be addressed. First, it may be argued that there is a potential sample bias because these patients were recruited from a tertiary referral centre for GTS, and could present with more severe forms of the disease and higher levels of comorbidity than the average GTS patient. However, the clinic assesses large numbers of patient in whom GTS is suspected in the United Kingdom; hence, they present with a range of symptom severity and associated behaviours.

A second criticism may relate to the effects of antidopaminergic drugs on FP-CIT binding. The study patients were all neuroleptic-naïve, except for one who had not taken any antipsychotic drug for more than a year before the scan. It is possible that patients may have unknowingly taken these drugs (i.e. prescribed by family doctors, etc.); however, we are very confident that this is not the case. It has also been reported that the effects of chronic treatment with neuroleptics does not significantly change the pattern of tracer binding to DAT in humans (39, 40) or rats (41, 42). None of the patients had a drug screen test, but the use of amphetamine or derivatives (both prescribed and non-prescribed) and of cocaine was ruled out by careful clinical assessment and history taking. None of the patients had taken any dopamine depleting drug ever.

A third criticism may relate to the effects of tic suppression and tic activity during scanning. No instructions were given to patients to suppress any abnormal movements during tracer injection and uptake. Image acquisition took place after the relatively irreversible accumulation of FP-CIT in the basal ganglia. Tic activity during scanning was minimal in all patients. Hence tic suppression during scanning was not expected to affect the results.

A fourth criticism could be related to the effects of ADHD on binding ratios. Five of the GTS patients had a history of childhood ADHD but none had current ADHD according to the DSM-IV (1) criteria. Furthermore, it has been reported that adult ADHD is not associated with abnormalities in DAT (43).

Finally, factors related to sample size needs to be taken into consideration. Although with ten subjects in each group, we have observed significant differences in striatal binding, as the sample size

ay not be large enough to show associations etween binding and measures of psychopathology.

In conclusion, in this study we have demonstrated that patients with GTS show increased striatal inding to DATs across both caudate and putamen a comparison with control subjects matched for ge and gender. However, it appears that other ehavioural and psychiatric symptoms that can ccur in GTS patients are not directly related to neasurable abnormalities in striatal DAT activity.

onflict of interest

None. The radiotracer was supplied free of charge y Nycomed Amersham plc., UK.

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ORIGINAL ARTICLE

A Functional Neuroanatomy of Tics in Tourette Syndrome

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Background: Tics are involuntary, brief, stereotyped motor and vocal behaviors often associated with irresistible urges. They are a defining symptom of the classic neuropsychiatric disorder, Tourette syndrome (TS), and constitute an example of disordered human volition. The neural correlates of tics are not well understood and have not been imaged selectively.

Methods: Event-related [^{15}O]H_2O positron emission tomography techniques combined with time-synchronized audio and videotaping were used to determine the duration of, frequency of, and radiotracer input during tics in each of 72 scans from 6 patients with TS. This permitted a voxel-by-voxel correlational analysis within Statistical Parametric Mapping of patterns of neural activity associated with the tics.

Results: Brain regions in which activity was significantly correlated with tic occurrence in the group included medial and lateral premotor cortices, anterior cingulate cortex, dorsolateral-rostral prefrontal cortex, inferior parietal cortex, putamen, and caudate, as well as primary motor cortex, the Broca's area, superior temporal gyrus, insula, and claustrum. In an individual patient with prominent coprolalia, such vocal tics were associated with activity in prerolandic and postrolandic language regions, insula, caudate, thalamus, and cerebellum, while activity in sensorimotor cortex was noted with motor tics.

Conclusions: Aberrant activity in the interrelated sensorimotor, language, executive, and paralimbic circuits identified in this study may account for the initiation and execution of diverse motor and vocal behaviors that characterize tics in TS, as well as for the urges that often accompany them.

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TOURETTE SYNDROME (TS) is a classic neuropsychiatric disorder characterized by multiple motor and vocal tics. Tourette syndrome is seen worldwide, with typical onset in childhood, and a prevalence of approximately 5 per 10000.[1] Comorbid obsessive-compulsive, attention-deficit, and learning disorder features have been described as well.[2] There is a significant genetic component in TS, with a suggestion of autosomal dominant transmission, although there have been no significant linkage findings to date.[1,3,4] Autoimmune mechanisms have also been implicated in some cases.[5,6]

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Tics, the defining symptom of TS, are sudden, brief, stereotyped actions. They may be simple vocalizations (such as grunting or sniffing), or movements of individual muscle groups. Alternatively, they may be complex in nature, comprising whole words (including curses [coprolalia]) or clusters of movements.[2] Mild tics can be unintentional, involuntary actions that can occur without a patient's awareness. However, the more severe or complex tics are often intentional, "unvoluntary" actions, in that they are briefly suppressible, performed to relieve a local tension, sometimes preceded or provoked by an uncomfortable sensation, or performed compulsively in association with irresistible urges.[2,7] In these cases, the subjective sense of free will is disrupted: tics are performed against the patient's will, or the will to act is not under the patient's control. Therefore, TS provides a model of one type of disordered human volition.

The neural correlates of these striking symptoms of volitional disruption are not well defined. Basal ganglia dysfunction has been suggested by the occurrence of tics in pathological conditions that affect these deep structures, such as carbon monoxide poisoning and encephali-

SUBJECTS AND METHODS

SUBJECTS AND CLINICAL CHARACTERIZATION

Six right-handed male patients with a *DSM-IV*[38] diagnosis of TS (mean age, 36.7 years, range, 25-47 years; duration of illness: mean±SD, 30.3±10.9 years) and frequent tics were studied after informed consent was obtained. Two patients were unmedicated and 4 suffered from tics despite neuroleptic medication (chlorpromazine dose equivalents, 2175, 50, 300, and 75 mg). All patients were assessed with the Yale Global Tic Severity Scale (mean±SD score, 39.3±12.2 of 55), the questionnaire form of the Leyton Obsessional Inventory (mean±SD score, 23.0±15.9 of 68), and the Beck Depression Inventory (mean±SD score, 7.2±6.0 of 39). Patients with notable head or neck tics, which could produce substantial head movement, were excluded. During the study sessions, all patients had simple and complex motor tics in varying muscle group distributions. Five patients had simple vocal tics, 4 of them had complex vocal tics, including coprolalia in 3.

SCAN CONDITIONS

Each of the patients was scanned 12 times, once every 10 minutes. Before each of the scans in the study session, each patient was instructed to relax, to close his eyes, and to allow the tics to emerge if they happened to occur, without any effort to induce or suppress them. The motor tics were monitored with 2 video cameras: 1 focusing just on the face and 1 covering the entire body. The vocal tics were monitored with a throat microphone and tape recorder, as well as with the video/audio camera. The video and audio tapes were time-synchronized with the computer that logged the whole-brain time-activity curve, which reflects radiotracer delivery to the brain during each scan. Head motion was kept to less than the full-width half maximum of the smoothed spatial resolution of the images with a custom-designed head holder that provided comfortable restraint.

IMAGE ACQUISITION, PROCESSING, AND STATISTICAL ANALYSIS

Regional cerebral blood flow was measured (as an index of neuronal activity) with a Siemens 953B PET scanner (Siemens Medical Systems, Hoffman Estates, Ill) in high-sensitivity 3-dimensional mode using a low-dose (15-mCi) [^{5}O]H_2O slow-bolus technique, with 90-second acquisition (including a critical period of approximately 30 seconds, during which the pattern of radiotracer distribution in the brain is determined).[35] The procedure was covered under an approval by the local hospital ethical committee and the Administration of Radioactive Substances Advisory Committee, United Kingdom. The data were corrected for background activity and attenuation, reconstructed (Hanning filter 0.5; 8.4-mm resolution full width half maximum), and the images were realigned to one another, smoothed with a 15×15×15-mm gaussian filter, transformed to the stereotactic space of Talairach and Tournoux,[39] and normalized using an analysis of covariance to remove the effect of differences in global blood flow across scans or sessions.[40] For each of the 12 scans in the study session, the type and distribution of each tic, and the timing and duration of each tic in relation to radiotracer delivery was noted on video and audio tapes. This information was obtained by a neuropsychiatrist (K.-Y.C.) extremely experienced with tics and TS patients, who repeatedly watched and listened to the tapes (which had time markers) to determine the tic information for each second of the critical radiotracer delivery periods. This information was then used to derive a weighted score for each scan,[36] reflecting the contribution of radiotracer deposition during tics to the image (**Table 1**). There was a spread of scan scores over the study session, reflecting differences across scans in the exact timing, duration, and frequency of tics, as well as the dynamic nature of the radiotracer input function during each scan. An event-related count rate correlational analysis[36] was then performed within the framework of voxel-by-voxel Statistical Parametric Mapping.[40] Without the need for separate "nontic control scans," this symptom-specific analysis identifies voxels with intensities covarying with the scores, corresponding to areas of the brain in which activity is specifically associated with the tics. The group analysis was performed for all 72 scans by determining the significance of the average covariate (tic scan score) effect, or average correlation, at each voxel, in a linear model (multiple regression with block effect), including subject-specific parameters for the scan scores, an effect for global cerebral blood flow, and additive subject effects; the latter adjust for between-subject differences in mean regional cerebral blood flow not accounted for by global changes. The effect of neuroleptic medication was minimized within subjects by focusing exclusively on the variance induced by tics (present despite medication in the medicated subjects) within a study session in which the dose was constant; it was minimized across subjects by the removal of subject-specific effects (which included medication dose), and by considering chlorpromazine equivalent doses as covariates of no interest. A similar, single-subject analysis was performed with data from an individual patient, for whom separate coprolalia/vocal tic and motor tic scores were calculated for each of 12 scans in the study session.

tis lethargica.[8,9] The dopaminergic system has been implicated in TS because dopaminergic medication can induce tics, while blockade of dopaminergic neurotransmission can be effective in their suppression.[2] Most in vivo radioligand imaging and postmortem histochemical studies of TS have therefore focused on presynaptic and postsynaptic dopaminergic function in the basal ganglia,[10-15] although a number of other brain regions and neurochemical (including peptide and second messenger) systems have been examined.[16-18] Recent structural magnetic resonance imaging studies have demonstrated abnormalities of volume and lack of normal asymmetry in the basal ganglia.[19-22] A possible role for the anterior cingulate and midbrain in the generation of tics has also been suggested.[23,24] Single-photon emission computed tomography and fludeoxyglucose F 18 positron emission tomography (PET) studies in the "resting" baseline state[25-33] have produced variable results, with decreased or increased activity described in regions such as the striatum and thalamus, and premotor, sensorimotor, and paralimbic cortices. Disordered interactions between subcortical, paralimbic, and sensorimotor brain regions have also been

postulated.[30] A recent functional magnetic resonance imaging study[34] focusing on the suppression of tics found that increased severity of tics outside the scanner was associated with less of a suppression-related decrease in ventral globus pallidus, putamen, and midthalamus activity (and less of a corresponding increase in midfrontal, lateral temporal, inferior occipital, and head of caudate activity).

To date, the functional neuroimaging experiments of TS have provided extremely valuable information, but have not measured (or, in some cases, controlled for) tic occurrence during scanning, and therefore have not generated an image of the brain state specifically associated with tics. We have developed and validated methods of PET image acquisition and analysis[35,36] that can isolate patterns of brain activity associated with transient, randomly occurring neuropsychiatric states. These methods have been used to study the functional neuroanatomy of hallucinations (involuntary perception) in schizophrenia.[37] In this study, they were used to examine the pathophysiology of tics (unvoluntary/involuntary action) in TS.

Table 1. Tic Scan Scores*

	Subject No.					
Scan No.	1	2	3	4	5	6
1	0.269	0.096	0.761	0.532	0.509	0.959
2	0.798	0.167	0.942	0.822	0.309	0.944
3	0.607	0.494	0.953	0.955	0.156	0.765
4	0.590	0.435	0.946	0.997	0.389	0.686
5	0.745	0.192	0.284	0.727	0.291	0.379
6	0.657	0.087	0.514	1.000	0.302	0.307
7	0.331	0.099	0.477	0.850	0.322	0.581
8	0.389	0.135	0.791	0.986	0.202	0.338
9	0.796	0.624	0.588	0.954	0.776	0.767
10	0.818	0.998	0.883	0.952	0.589	0.998
11	0.588	0.576	0.887	0.826	0.859	0.998
12	0.953	0.621	0.866	0.771	0.849	0.999

**Scan scores represent the radiotracer deposition during motor and vocal tics, hence the contribution of these events to the resultant image. These weighted scores constitute the covariate of interest in the group correlational analysis performed. See the "Image Acquisition, Processing, and Statistical Analysis" subsection of the "Subjects and Method" section, as well as Silbersweig et al.[36]*

RESULTS

The group results were assessed at a threshold of $P<.005$, with spatial extent of activations corrected for multiple comparisons at a threshold of $P<.05$. Increased brain activity highly correlated with tic behavior was detected in a set of neocortical, paralimbic, and subcortical regions, including supplementary motor, premotor, anterior cingulate, dorsolateral-rostral prefrontal, and primary motor cortices, the Broca's area, insula, claustrum, putamen, and caudate. Activations in superior temporal gyrus, inferior parietal cortex, and a point near the anterior thalamus, extending toward the head of caudate were also detected. These foci of activation are displayed and characterized in **Figure 1** and **Table 2**.

For a single subject in whom greater than 90% of vocal tics were coprolalia, separate scan scores were generated for coprolalia/vocal tics and for motor tics. At a threshold of $P<.005$, with spatial extent corrected for multiple comparisons at $P<.05$, coprolalia was associated with activity in a set of regions, including the frontal operculum and the Broca's area (Brodmann areas [BAs] 44, 45) ($z=4.34$; $x, y, z=-30, 20, 16$) (extending ventrally in the opercular region adjacent to BA 47; $z=2.88$; $x, y, z=22, 28, 0$); superior temporal gyrus (BA 42; $z=4.22$; $x, y, z=-60, -26, 8$) (BA 22; $z=4.05$; $x, y, z=-50, -40, 12$); head of caudate ($z=3.46$; $x, y, z=-4, 10, 12$); body of caudate ($z=2.72$; $x, y, z=12, 6, 20$); a region near the tail of the caudate and hippocampus ($z=3.67$; $x, y, z=34, -40, 4$); supramarginal gyrus (BA 40; $z=3.43$; $x, y, z=-54, -50, 32$); posterior insula ($z=3.37$; $x, y, z=30, -26, 4$); middle temporal gyrus (BA 21; $z=3.31$; $x, y, z=-54, -60, 0$); putamen ($z=3.29$; $x, y, z=-16, 16, 4$); cerebellar vermis ($z=3.23$; $x, y, z=4, -54, 0$); cerebellum ($z=2.77$; $x, y, z=12, -48, -12$); posterior thalamus ($z=2.98$; $x, y, z=22, -26, 8$); medial thalamus ($z=2.78$; $x, y, z=-6, -12, 16$); and posterior cingulate gyrus ($z=2.82$; $x, y, z=6, -44, 8$). At the same threshold, motor tics were associated with activation in a region deep to inferior parietal and sensorimotor cortex ($z=5.04$; $x, y, z=-40, -22, 28$); sensorimotor cortex (BAs 2, 4; $z=3.96$; $x, y, z=-48, -22, 36$); superior temporal gyrus (BAs 42, 22; $z=3.60$; $x, y, z=-56, -26, 16$); and somatosensory cortex (BA 2; $z=2.70$; $x, y, z=-60, -22, 24$) (**Figure 2**). While the sensorimotor activations were on the left at this threshold, right-sided activations, as well as putamen activation, were seen at a lower threshold ($P<.01$, uncorrected) in this subject. It should be noted that, although the distribution differed, vocal tics occurred simultaneously with a subset of the motor tics in this subject, so one would not expect that these activations represent maps purely of vocal or motor tics. It should also be kept in mind that certain regions of activation may become apparent with the increased power of the group analysis, while other regions may be more specific to an individual analysis.

COMMENT

These results define a distributed neural system in which abnormal activity is associated with the spontaneous initiation of, or failure to suppress, motor and vocal behavioral repertoires in this group of TS patients. Prominent activity was noted in primary motor and Broca's areas, corresponding to the modality-specific outflow pathways of behavioral expression in motor and vocal tics. Striatal activity was also noted, supporting the involvement of basal ganglia circuits that are emphasized in traditional pathophysiological models of TS. The extensive activity in executive and premotor regions may be particularly notable, and may help to extend our understanding of disordered action and volition in TS, because these regions have traditionally been associated with the selection, preparation, and initiation of behavior.

Activity detected in the striatum can be seen in the context of cortico-striato-pallido-thalamo-cortical circuits that modulate activity in parallel brain systems underlying discrete psychomotor functions with specific functional and somatotopic organization.[41] Within these circuits, the direct and indirect basal ganglia pathways provide a balance of excitation and inhibition[42] that may

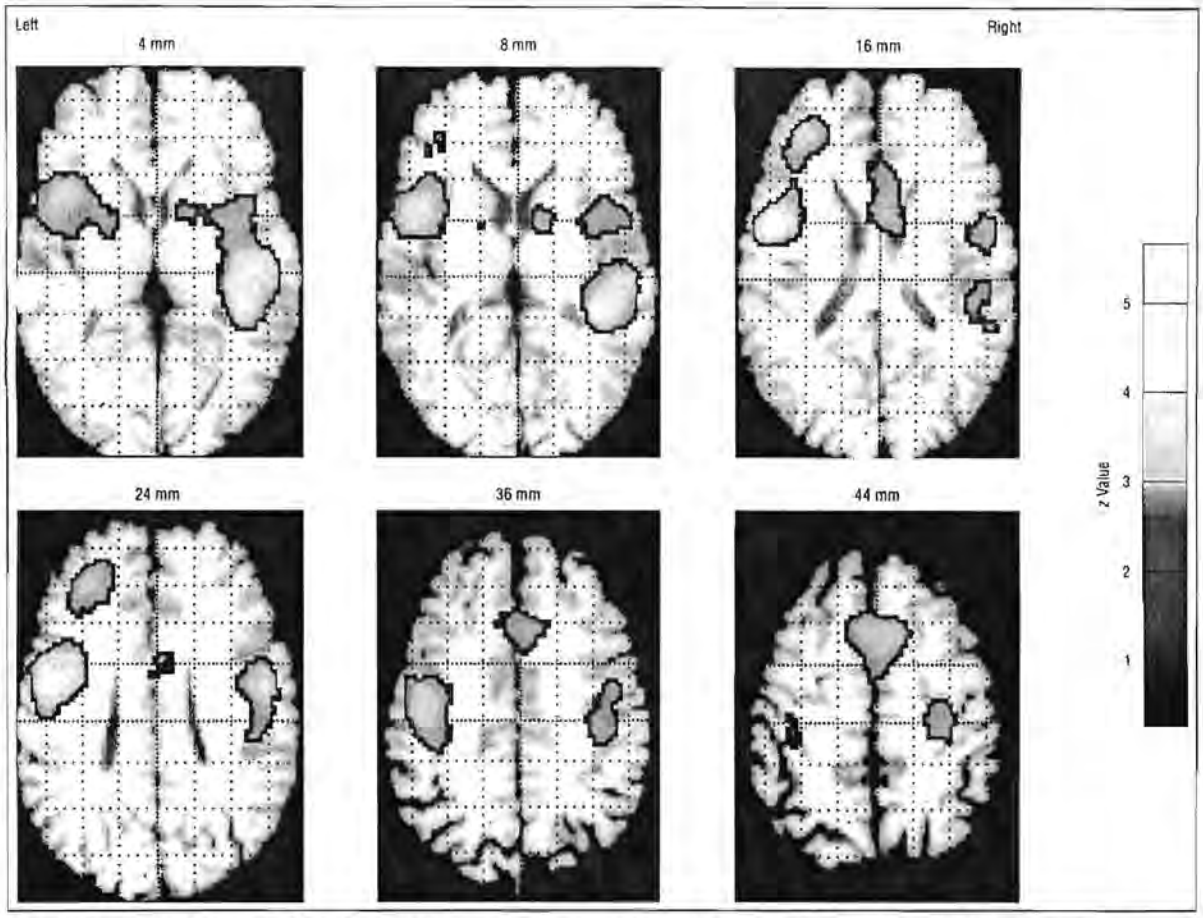

Figure 1. *Stereotactic axial sections of brain areas with significantly increased activity during tics (motor and vocal) in 6 patients with Tourette syndrome. Functional positron emission tomography results (thresholded at P<.005, with spatial extent corrected for multiple comparisons at a threshold of P<.05) are displayed in color, superimposed on a single structural T1-weighted magnetic resonance imaging scan that has been transformed into the stereotactic coordinate space of Talairach and Tournoux[39] (for anatomical reference). Section numbers refer to the distance (in millimeters) from the anterior commissure–posterior commissure line, with positive numbers being superior to the line. The areas of maximal activation are described in the text and listed in Table 1.*

be disrupted in TS. A failure of inhibition in motor cortex of TS patients, due to subcortical afferent disinhibition and/or to failure of intracortical inhibition, has been suggested by a transcranial magnetic stimulation study.[43] The findings of the current study implicate 3 of the cortico-striato-pallido-thalamo-cortical circuits in particular: the motor, dorsolateral prefrontal, and anterior cingulate circuits. These circuits are involved in the selection, programming, initiation, and control of movement.[44] Dopaminergic projections from the midbrain tegmentum, a region where activation was noted at a threshold of $P<.005$ (uncorrected), are involved in the modulation of these circuits.[41] This modulation may provide a mechanism of symptom formation (excess dopamine) and treatment effect (dopamine blockade) in TS.[2]

For particular tics, the specific cortical and subcortical regions that are activated may determine the phenomenology of the behavior. In the individual analysis, coprolalia (which comprised more than 90% of the vocal tics) was associated with activation in the region of the Broca's area and the frontal operculum, known to be involved in the generation of speech. Activation was also noted in the head of the caudate, which has recently been identified by lesion methods as a critical component of the network underlying language.[45] The other language regions noted (including posterior superior temporal gyrus, middle temporal gyrus, and supramarginal gyrus) may have been involved in the generation or the subsequent hearing of the self-generated linguistic material. Activation in the posterior congulate gyrus has recently been described in association with emotional linguistic material.[46] The thalamic and cerebellar activations are consistent with the roles of these structures in modulating outflow of the cortical-subcortical circuits implicated. In contrast to the vocal tics, motor tics were associated with notable sensorimotor cortex activation. It is likely that activations of somatotopically specific subregions in sensorimotor cortices would be associated with movements in specific corresponding muscle groups.

Activity in anterior cingulate, premotor, and supplementary motor areas, and dorsolateral prefrontal cortex, detected in the current study, has been described in tasks involving conscious, volitional behavior, and is thought to be involved in the selection, preparation, and initiation of action.[23,47-52] Activity in the supplementary motor area has also been noted in the performance of over-

learned (automatic) motor sequences.[53] Activation of medial premotor association cortices has been associated with self-generated movements and activation of lateral premotor association cortices has been associated with externally cued voluntary movements.[54] The striking activation of both medial and lateral premotor systems in this study suggests that both systems can be implicated in "unvoluntary" internally generated action. The involvement of the lateral premotor system may reflect the response to internal sensations, which are now known to be a common component of tics in TS.[55,56] One study has reported a lack of normal premovement potentials associated with simple tics,[57] while another study found that premotor potentials were present during tics in some patients.[58] In either event, tics may differ from externally cued, planned movement in the timing, sequence, coherence, or distribution of premotor activity, and complex tics might be expected to involve more premotor activity than simple tics. A study of electroencephalogram microstates suggested differences between TS patients and normal subjects during simple and complex movements.[59] While the purpose of this study was to characterize the functional neuroanatomy of tics, future comparisons of tics vs volitional movements in TS patients, and of volitional movements in TS patients vs normal subjects, may help to clarify these issues.

The lesion and stimulation literature is also relevant to the interpretation of the findings in this study. Lesions or failure of activation of the medial frontal premotor system, prominently activated in this study, have been associated with the inability to initiate voluntary action.[60,61] Conversely, stimulation of, or seizure activity in, these regions can produce complex vocal and motor automatisms (sometimes associated with urges and emotions) resembling tics. This is particularly the case with the anterior cingulate, which is part of the rostral limbic system, and integrates affective cues with executive functions for the selection of context-dependent behavior.[23,62] The prominently activated insula is also involved in the integration of internal motivational states, with behavior appropriate for the extrapersonal world (entailing behavioral triggering or inhibition functions), in the imparting of affective tone to experience and behavior, and in somatosensory, linguistic, and self-generated motor functions.[63] Like the cingulate, it performs these roles by serving as a convergence point with widespread multimodal, limbic, and basal ganglia connections.[63] Dysfunction (including abnormal gating) in these phylogenetically older paralimbic regions may contribute to the primitive, uninhibited behavior of TS. The maxima of some of the activations in the insular region were centered on the claustrum. While such a small localization must be considered with caution, it is worth noting that the claustrum has connectivity with sensorimotor, premotor, and anterior cingulate regions, and is involved in the performance of movements.[64-66]

The predominantly dorsal location of anterior cingulate activation, rostral location of supplementary motor activation,[67] and dorsolateral location of prefrontal activation associated with tics in this study represents intermittent increased activity of executive components of the motor system (although supplementary motor cortex overall is considered premotor, and the cingulate also contains direct corticospinal projections[23]). Executive dysfunction has been noted in neuropsychological tests of patients with TS.[68,69] Tonic overactivity of frontal executive systems, coupled with hypoactivity in primary sensorimotor cortices, has been implicated in idiopathic dystonia, characterized by involuntary motor posturing and slowing.[70] Increased activity in orbitofrontal cortex and anterior cingulate cortex, and their subcortical connections (including the head of the caudate), has been implicated in obsessive-compulsive symptomatology,[71] characterized by involuntary thoughts and complex actions, and seen with increased frequency in patients with TS.[2] Given the differential prefrontal projections to various regions of the striatum (premotor to putamen and prefrontal to head of caudate), it might be expected that putamen dysfunction would be associated with a greater degree of motor symptomatology, whereas caudate dysfunction would be associated with a greater degree of cognitive symptomatology.[72]

The results of this "state" study of tics in TS may also be seen in the context of prior "trait" studies[25-31] of TS. When the variable results of the previous trait studies are taken together, they suggest a tonic dysregulation of a number of the regions in which increased activity was detected in this symptom-state examination.

Table 2. Local Statistical Maxima in the Pattern of Brain Activity During Tics in 6 Patients With Tourette Syndrome

Region (Brodmann Area)	x, y, z*	z Score
Left		
Precentral gyrus (4)	−46, −12, 28	5.82
Precentral gyrus (lateral 6)	−46, −4, 20	5.33
Middle frontal gyrus (lateral 6)	−44, 4, 12	4.93
Middle frontal gyrus (10)	−32, 40, 20	4.32
Superior frontal gyrus (9)	−26, 36, 28	3.76
Middle frontal gyrus (9/46)	−32, 32, 24	3.72
Claustrum/insula	−30, 10, 0	3.56
Inferior frontal gyrus (45)	−38, 26, 16	3.23
Putamen	−22, 2, 0	3.17
Inferior parietal lobule (40)	−34, −32, 40	3.02
Anterior cingulate gyrus (32)	−22, 20, 28	2.74
Right		
Precentral gyrus (4/6)	46, −8, 28	4.91
Caudate (tail), posterior insula†	36, −38, 4	4.72
Superior temporal gyrus (22)	46, −22, 4	4.72
	48, −36, 8	3.70
Insula	38, −26, 0	4.56
	38, 4, 8	3.13
Medial frontal gyrus (6)	0, 14, 44	4.43
Anterior cingulate (24)†	2, 4, 20	3.91
Anterior cingulate (32)	8, 16, 40	3.86
Inferior parietal lobule (40)	42, −28, 28	3.76
Caudate (head)	4, 12, 16	3.58
Postcentral gyrus (3)	28, −24, 44	3.47
Anterior thalamus/caudate (head)	10, 0, 8	3.26
Claustrum/insula	34, −2, 4	3.24

**Coordinates in millimeters relative to the anterior commissure: x is the lateral distance from the midline (positive = right); y, the anteroposterior distance from the anterior commissure (positive = anterior); and z, the height relative to the intercommissural line.*

†Indicates the maximum coordinate in a brain region adjacent to a gray matter structure that is part of the same contiguous activation.

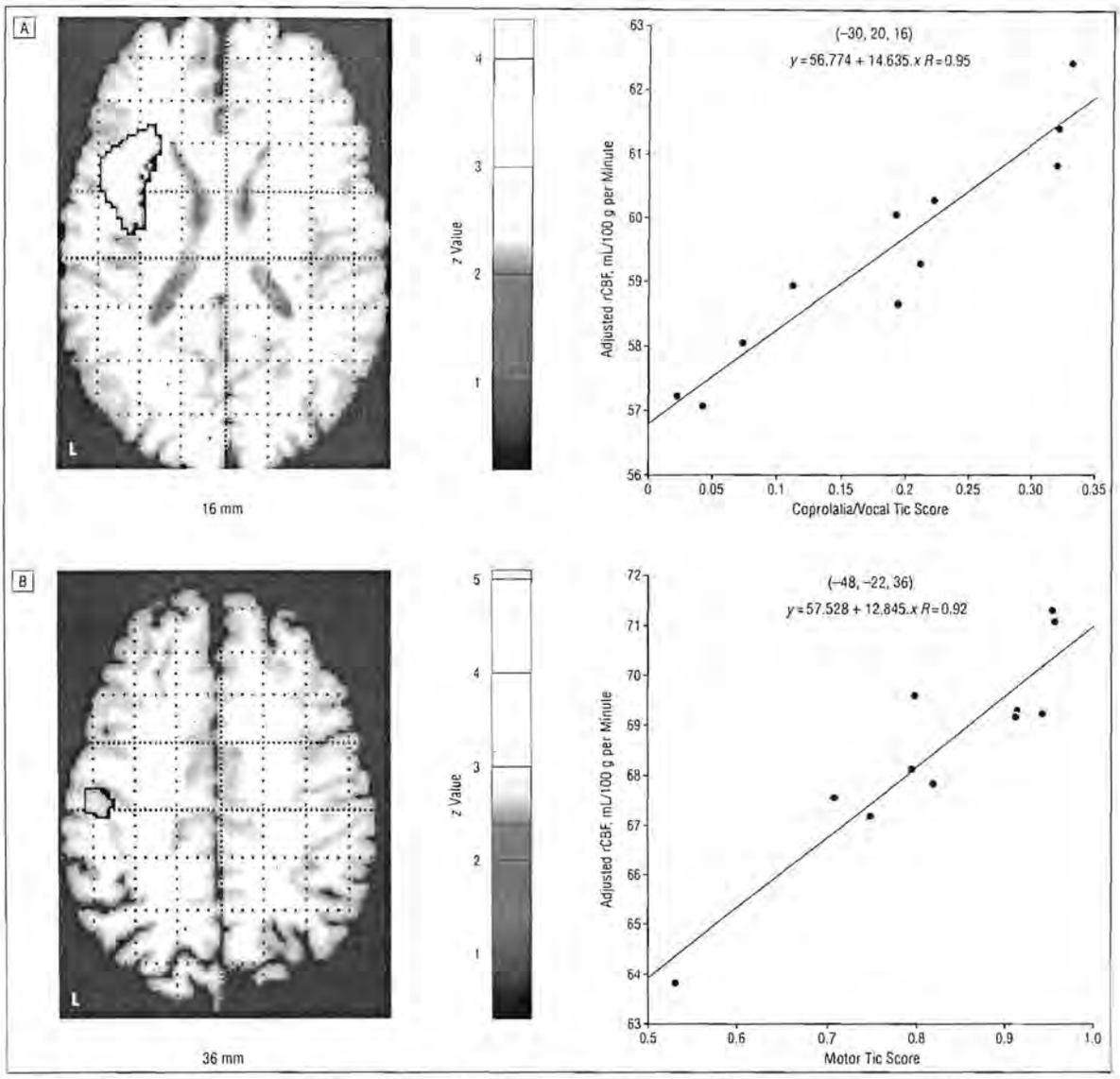

Figure 2. *Correlations in a single patient with Tourette syndrome between regional cerebral blood flow (rCBF) and scan scores for coprolalia in the left frontal operculum/ inferior frontal gyrus (A), and for motor tics in the left sensorimotor cortex (B). These activated regions (thresholded at P<.001, with spatial extent corrected for multiple comparisons at P<.05) are superimposed in color on axial slices from a stereotactically transformed structural magnetic resonance imaging template. L indicates left.*

The decreased activity noted in some of the previous studies may reflect inhibition of tics during those study sessions[31] (such inhibition is unlikely in the current study, as patients were reminded not to supress tics before each scan and had frequent tics during each scan, with which brain activity was directly correlated). It is also possible that tonic decreased activity alternates with intermittent increased activity during tics in the regions implicated in TS. Although tics are not frank seizures, such a temporal pattern would be similar to that described in PET studies of epileptic foci,[73] and consistent with an imbalance of excitation and inhibition.

The results of this study may help to expand the interpretation of the results of a previous functional magnetic resonance imaging study that examined tic suppression in TS.[34] That study compared a condition in which tics were suppressed with a condition in which tics were expressed. The authors interpreted their findings with an emphasis on the issue of suppression, and make the reasonable suggestion that failure to inhibit tics in TS may result from an impaired ability to alter subcortical neuronal activity. While they noted that the higher rate of spontaneous tics in their control condition was a possible confounding factor, they felt that this was unlikely because they expected that the higher rate of tics would produce a greater change in magnetic resonance imaging signal intensity during successful tic suppression, and correlate positively (not negatively, as observed) with severity of tic symptoms (measured outside of the scanner). However, this would not be the case in regions active during both tics

and (possibly to a different degree in) their suppression. In the current study, tics were not suppressed, and were counted and characterized during the scans and differences in numbers of tics, and possibly urge, are not an issue. The results of these 2 studies can therefore be taken together, possibly suggesting that anterior cingulate and midfrontal activity is common to both tics and their suppression, and that putamen and sensorimotor cortex (motor outflow) activity is higher during tics and lower during suppression. While the pattern of increased activity noted in the current study could be primary, it is also quite possible that it could result from failure of inhibition.

Although these statistically significant results represent a sampling of hundreds of tics in 72 images from multiple subjects, the population studied is still relatively small, the analysis applies for just this group of subjects, and further studies will be necessary to replicate, extend, and assess the generalizability of these findings. Possible medication effect in 4 of the subjects also has to be considered as a potential limitation, although a number of points make this issue less likely to affect the results: the target symptom (tics), and therefore the neural firing underlying it, was active despite medication in the 4 medicated patients (2 were unmedicated); the analysis determines the variance induced by tics in a constant pharmacodynamic/pharmacokinetic setting over the course of the study session; and subject-specific effects, including medication and dosage (chlorpromazine equivalents), were partialed out in the analysis. Despite the 2 videotapes and throat microphone, it is possible that extremely subtle tics may have been missed, although patients with known severe, stereotypical tics were studied. Regarding timing, it should be kept in mind that the temporal discrimination achieved with this technique is not due to direct temporal resolution, the timing measures were to the nearest second, possible subcomponents of tics cannot be resolved, and it is not possible to say where the activation in the identified systems begins, or whether it occurs in parallel. Future analyses and studies can examine these issues, as well as similarities and differences between subtypes of tics, within and between individual unmedicated patients.

In conclusion, activity was noted specifically during tics in motor/vocalization, paralimbic, premotor, and executive frontal-subcortical brain systems. Autonomous activity in these regions may account for the striking motor and vocal acts of TS patients, and may contribute to the "unvoluntary" experience of an irresistible urge that often accompanies these acts. Indeed, tics may represent a paradoxical state in which brain regions important for motivational aspects of behavior, and normally associated with a subjective sense of volition as they initiate action, are not operating under the volitional control of the patient. This suggested systems-level pathophysiology of TS is consistent with observations of behavioral changes associated with lesions and stimulation in medial frontal regions, and these findings may contribute to a framework for future studies.

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A volumetric MRI study of Gilles de la Tourette's syndrome

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Article abstract—The neuroanatomic or neuropathologic basis of Gilles de la Tourette's syndrome (GTS) remains unknown. Recent studies have suggested abnormalities of cerebral asymmetry and basal ganglia volumes. We studied 17 patients with GTS and eight normal controls using volumetric MRI techniques for measuring the caudate nucleus, amygdala, and corpus callosum. One subject with GTS was subsequently excluded because he was left handed. No absolute differences in caudate nucleus volumes between patient and control groups were evident. There was an increase in corpus callosum (CC) cross-sectional area and a loss of the normal asymmetry of the caudate nucleus in the patient group. A loss of the normal correlation between cross-sectional area of the CC and whole brain index (WBI) in the patient group also was found. The amygdala measurements had a poor interrater reliability.

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Gilles de la Tourette's Syndrome (GTS) is a disorder defined by the presence of multiple motor and one or more vocal tics with childhood onset and a fluctuating course.[1,2] It is further characterized by the presence of symptoms such as coprolalia and echolalia. Genetic[3,4] and epidemiologic[5,6] studies have shown GTS to be associated with obsessive compulsive disorder. The precise etiology of GTS remains unknown, but it is thought to be an autosomal dominant genetic disorder.[4]

The neuropathologic basis of GTS also is unknown. Postmortem pathologic studies of patients with GTS suggested reduced dynorphin in the lateral globus pallidus[7] and neuronal hypoplasia in the caudate nucleus (CN) and putamen.[8] Cases of "secondary GTS," in which the symptoms of the syndrome are seen to develop following identified brain lesions in persons without any family history of GTS,[9,10] have also shown involvement of the basal ganglia and medial temporal structures. It is therefore reasonable to assume that dysfunction of the basal ganglia and medial temporal lobe may be the basis of the clinical syndrome of GTS.[11] Neuroimaging techniques offer us the prospect of examining such hypotheses.

Single photon emission computed tomography (SPECT) studies have found regional cerebral blood flow (rCBF) abnormalities in the striatum and medial temporal areas.[12-14] PET studies have revealed metabolic dysfunction in the striatum and frontal, cingulate, and insular cortices.[15-16] MRI studies[17-19] have found subtle anatomic abnormalities in the lenticular and caudate nuclei using volumetric MRI methods. These studies support neuroanatomic data implicating the basal ganglia in the clinical syndrome of GTS. However, they also raise important questions, which we propose to address. The hypofunction of medial temporal and anterior striatal areas, observed in previous studies using functional imaging techniques, might relate to underlying structural abnormality. The present study is a morphometric study of the CN and amygdala using MRI volumetric techniques. We also examine whether the changes to normal cerebral asymmetry reported by other investigators are seen in the CN and amygdala.

In addition, Witelson[20] has argued that the consistency in loss of asymmetry in basal ganglia structures is compelling, and she suggests that measurement of size of the CC may be illuminating in the study of GTS, given that this structure appears to be a sensitive indicator of cortical anatomy.[21] Other studies have to date produced conflicting results in the measurement of CC size with both increases[22] and decreases[23] having been described. Therefore, we measured the cross-sectional area of the CC to determine whether there is any difference in the size of the CC in patients with GTS compared with normal controls.

Methods. Twenty-five scans were performed on 17 patients (11 male, six female) and eight normal controls (four male, four female). The mean age of the patients was 35 years, (SD,13; range, 17–62); that of the controls 33 years, (SD, 10; range, 20–45). All patients were recruited from consecutive referrals to the GTS clinic at the National Hospital for Neurology and Neurosurgery, Queen Square, London. All subjects were drug free for at least 3 months prior to the scan. One patient and none of the controls was

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left handed, as determined by exclusive preference for the right hand on all 12 items of the Annett handedness questionnaire.[24] This patient (a 35-year-old man) was not included in subsequent analyses.

MRI. All scans were acquired using a GE 1.5 tesla scanner with an identical protocol: (1) *Sagittal* 90° fast multiplanar spoiled gradient acquisition in steady state (SPGR), TR/TE 131/3.4, 12 images, 256 × 192 matrix and two excitations, 5 mm thickness; (2) *Axial* fast dual echo multiplanar acquisition TR/TE1/TE2 2000/30/75, 256 × 192 matrix, two excitations, thickness 5 mm, 40 images; (3) *Coronal* volumetric SPGR, TR/TE 35/5, flip angle 35°, 256 × 158 matrix, one excitation, 124 images, 1.5 mm thickness. The last sequence was used for volumetric analysis because of the small slice thickness and large number of available slices through the structures of interest, allowing the most appropriate sampling rates to be selected according to described stereologic principles. This protocol is identical to that described elsewhere for the volumetric analysis of the hippocampus in patients with localization-related epilepsy undergoing presurgical evaluation.[25] There is no interslice gap for these images.

Analysis. The settings for the gray-scale window and level were chosen visually to maximize the contrast between gray and white matter. For the amygdala, at ×1 magnification, the most anterior slice to be measured was identified as that in which the anterior commissure was visible ipsilaterally. The images were magnified by a factor of 3. Measurements proceeded from the posterior aspect of the amygdaloid complex. Reference was made to a standard neuroanatomic atlas throughout.[26] The criteria used to define the amygdaloid complex were adapted from those described by Watson et al.[27] Posteriorly, the inferior horn of the lateral ventricle was used to identify the separation of the amygdala from the hippocampus. The superior limit of the region of interest was consistently defined by a straight line joining the most lateral aspect of the entorhinal sulcus and the most inferior aspect of the circular sulcus of the insula. The gray matter–CSF boundary was used to identify the medial boundary of the region of interest. Inferolaterally, the wall of the uncal cleft of the inferior horn of the lateral ventricle was used as a boundary. The white-gray interface provided the more anterior lateral limit. The lateral and medial ends of the hippocampal-amygdaloid articulation were taken as the most medial aspect of the inferior horn of the lateral ventricle and the gyrus ambiens respectively. If, as was the case in some slices, the hippocampal-amygdala articulation could not be clearly seen, a straight line was used to join these two points. Otherwise, the articulation was traced manually along the line of the alveus. In cases in which the gyrus ambiens was not visible, a horizontal line was drawn from the most medial aspect of the inferior horn to the crural cistern, to define the inferior limit of our region of interest (figure 1).

The CN was measured bilaterally from its appearance anteriorly for a maximum of 20 slices (30 mm) (figure 2). As can be seen from the figure, the separation of the CN from the nucleus accumbens (NA) is not possible visually, and so when we speak of CN, we include part of the NA in that structure.

For the CC, the midline sagittal section was used, identified as the section that included the pituitary stalk, and

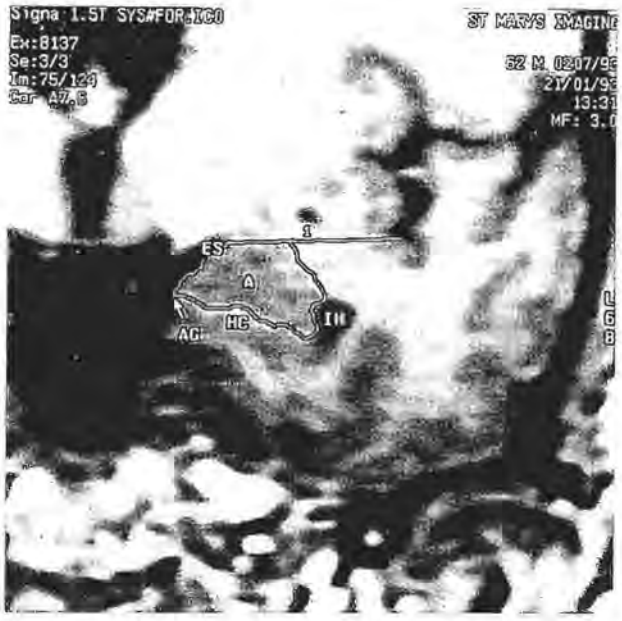

Figure 1. Coronal section with circumscribed region of interest for left amygdala (300% magnification).

cross-sectional area measured by tracing manually along the border of the CC (figure 3).

In addition, a measure to correct for whole brain volume was calculated using Peterson's method.[17] This index of brain volume, which is a morphometric approximation, is a product of three distances in three dimensions. The vertical distance is that of a line joining the inferior border of the mammillary body to the vertex perpendicular to a line joining the most anterior point of the genu of the CC to the most inferior point of the splenium. The anteroposterior and transverse diameters are perpendicular in a transaxial section at the level of the inferior border of the CC.

Reliability measures were based on two independent observer measurements (different observers at different

Figure 2. Coronal section with circumscribed region of interest for left caudate nucleus (300% magnification).

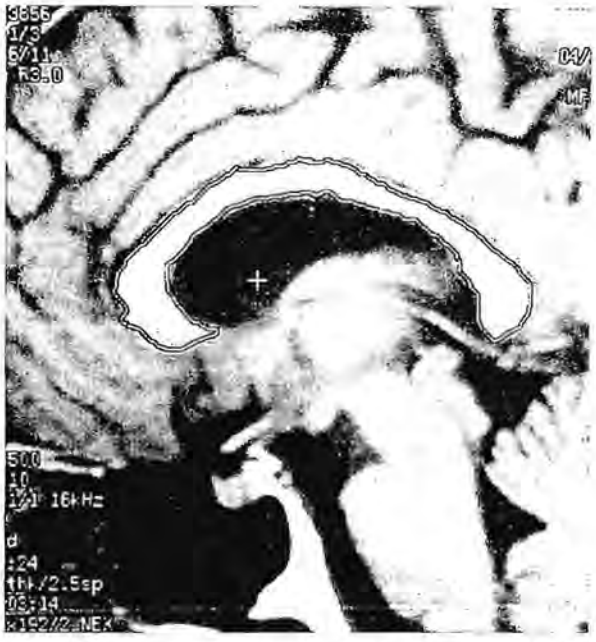

Figure 3. Midline sagittal section with circumscribed region of interest for corpus callosum (200% magnification).

times) on all 17 patients calculated as an intraclass correlation coefficient (ICC).[28] Three raters in all were used (J.M. and M.F. for the amygdala and J.M. and A.V. for the CN). Raters were blind to subject characteristics.

For the CN and the amygdala, an asymmetry index (AI) was calculated according to the formula

$$AI = \frac{(Right - Left)}{(Right + Left)}$$

Differences between groups in volume or AI were tested using one-way ANOVA with correction for multiple comparisons. Correlations between WBI and CC cross-sectional area were calculated (Pearson's product-moment).

Results. The ICC for the amygdala measurements was 0.47 on the right and 0.38 on the left. For the CN, 0.84 on the right and 0.91 on the left. The coefficient for both the WBI and the CC area was 0.96.

Mean volumes for the amygdalae are not reported here because of poor interrater reliability. Mean volumes for the CNs and the values for the WBI and callosal cross-sectional area are shown in table 1. There were no volume differences between GTS and controls for the amygdala or the CN, nor any difference in WBI between the two groups. Area of the CC was significantly greater in patients than in controls. Correcting the volume of the subcortical nuclei or CC for WBI did not affect the results.

Table 1 Mean (standard deviation) for volumetric measurements of caudate nucleus and for callosal cross-sectional area and whole-brain index

	GTS (n = 16)	CN (n = 8)
Right caudate	2,916 (385) mm^3	3,134 (525) mm^3
Left caudate	2,947 (420) mm^3	2,958 (473) mm^3
Corpus callosum	641 (57) mm^2	564 (67) mm^2 $p = .016$
WBI	1.67 (.17)	1.60 (.15)

GTS = Gilles de la Tourette syndrome; WBI = whole brain index.

Table 2 Pearson product-moment correlation coefficients for age and callosal cross-sectional area

Clinician group	N	ρ
Male	14	.01
Female	10	.19
Total	24	.08
GTS male	10	.19
GTS female	6	.70
GTS total	16	.34
NC male	4	−.67
NC female	4	−.73
NC total	8	−.65
(All not significant)		

GTS = Gilles de la Tourette's syndrome; NC = normal control.

Values for the asymmetry index for the amygdala are not reported, given the poor interrater reliability of the measurements. For the CN the mean asymmetry index is 0.00 (SD 0.01) for the GTS patients and 0.03 (SD, 0.01) for the normal controls, $p = 0.05$. Thus, there is a significant loss of normal right-side predominance of the CN in the patient group. If we divide our groups according to sex, we find the same patterns. The mean AI for male controls is 0.024, and for female, 0.033. The mean AI for the 10 male GTS patients is 0.005, and for the six females, 0.018.

A correlation exists between callosal cross-sectional area and WBI seen in normal controls ($\rho = 0.86$, $p < 0.01$). This is absent in the patient group ($\rho = -0.35$, NS).

No correlation was seen between age and CC cross-sectional area in men or women considered separately, or in the group as a whole (table 2). A decline in the cross-sectional area of CC with age is suggested in the normal controls but not in the patient group. This correlation is not significant.

Discussion. Most evidence from existing literature points to the involvement of basal ganglia and limbic structures (orbitofrontal, cingulate, insular, and medial temporal cortices) in GTS. Studies to date have not, however, clarified whether these findings are merely functional or linked to structural abnormalities of the same areas.

In our previous studies of patients with GTS using SPECT, we found reduced rCBF in the anterior striatum and left medial temporal areas.[12] Similar findings have been reported by other groups. Riddle et al.[13] found left striatal hypoperfusion in nine patients whereas Kurlan's group[14] found similar rCBF abnormalities in the right striatum in six. Chase et al.[15] described a PET study of 12 untreated patients (10 men and 2 women). No apparent abnormalities were found on visual inspection of the scans but quantitative analysis showed reductions in frontal, cingulate, and possibly, insular cortices and in the

*Table 3 Findings from structural imaging in Gilles de la Tourette syndrome**

Study	N	Findings
Caparulo et al. (1981)[38]	16	Ventricular enlargement
Lees et al. (1984)[39]	53	Normal
Harcherik et al. (1985)[40]	19	No characteristic differences
Regeur et al. (1986)[41]	53	5 Varied abnormalities
Chase et al. (1986)[15]	9	2 Minor abnormalities
Robertson et al. (1988)[42]	73	2 Cavum septum pellucidum
Demeter (1992)[43]	10	2 Focal abnormalities of basal ganglia
Case studies		
Yeragani et al. (1983)[44]	1	? Calcification of the caudate nucleus
Shaenboen et al. (1984)[45]	1	Enlargement of occipital horns lateral ventricles
Lakke and Wilmink (1985)[46]	1	Pineal tumor
Kjaer et al. (1986)[47]	1	Porencephalic cyst
Sandyk (1988)[48]	1	Asymmetric cerebral peduncles
Robertson et al. (1990)[49]	1	Right globus pallidus

* This list does not include the studies by Peterson et al.,[17] Singer et al.,[18] and Hyde et al.,[19] which are referred to in more detail in the text.

inferior corpus striatum. There was an inverse correlation between the severity of both vocal and motor tics and glucose utilization rates in these areas. More recently, this group has described abnormalities in terms of abnormal associations between metabolic rates in sensorimotor cortex and limbic areas.[16] Thus, consensus is emerging from the functional neuroimaging literature that patients with GTS show reduced CBF and metabolism in limbic structures.

Before 1993, no detailed structural imaging studies in GTS were done (table 3). CT studies found no characteristic abnormalities in the disorder or only minor abnormalities of doubtful significance, such as cavum septum pellucidum, porencephalic cyst, or asymmetry of cerebral peduncles. More recent studies, using sophisticated MRI morphometric methodology, have found more specific structural abnormalities. Two complementary papers in 1993 described reduced lenticular volume and a loss of the usual left-sided predominance of the lentiform nucleus in both adults[17] and children[18] with the syndrome. In the adult study, right-sided predominance of the CN was seen in both GTS and normal controls. In the study involving children, no significant CN asymmetry was found in either controls or patients. The possible role of the CN is again raised by Hyde et al.[19] This group studied monozygotic twin pairs discordant for severity of GTS and found reduced right CN volume in the relatively more severely affected twin as well as a loss of the normal ventricular asymmetry.

We found a loss of the normal CN asymmetry in patients with GTS. This is consistent with the report by Hyde et al.[19] In the study by Peterson et al.[17] of fourteen right-handed adults, there was a loss of the normal left-sided predominance of the lenticular nucleus. However, unlike us, they did not find a loss of the normal right-sided predominance of the CN in their GTS group. Singer[18] reported findings similar to those of Peterson et al. In their study, a male-only subgroup showed a trend towards absolute reduction in left lenticular volume. Although in our study we found no such absolute reduction in CN volume, the loss of asymmetry would seem to relate primarily to reduction in volume of the right CN. Our previous SPECT study[12] found decreased rCBF to be more marked in the left CN. SPECT measures are semiquantitative and only an indirect indicator of neural metabolism. Furthermore, the CN region of interest using SPECT does not exactly reflect the anatomic CN. Differences of resolution between the two techniques (the resolution of SPECT is considerably lower) are also present. Thus, if the CN was smaller, the fixed region of interest method using SPECT would include adjacent structures. However, even if this were the case, we would expect the effect would be to show an apparent reduction in blood flow to what was the smaller structure. The findings with functional imaging, especially in the light of recent MRI literature (including the present study) therefore need further elucidation. Coregistration of MRI and SPECT images or statistical parametric maps derived using PET would be most helpful in this endeavour.

Although the CN is primarily involved in the mediation of motor behavior, there is extensive evidence of its role in other complex behaviors.[29] Unilateral CN hemorrhage has been associated with amnesia, abulia, language disturbances, hallucinations, and varying degrees of apathy and disinhibition. These disturbances are reminiscent of frontal lobe disorders and indeed the cortical afferents to the striatum maintain the topographic representation of the cortical areas of origin. It would be inappropriate to try to account for the neurobehavioral manifestations of GTS solely with reference to the caudate itself. As already discussed, abnormalities of cerebral asymmetry and lateralization have been found by others in other structures and are likely to continue to be found in brain areas not at present amenable to volumetric MRI measurement.

We were aware from the outset that measurement of the amygdala on MRI images is difficult. The criteria described in the methods section were adapted from previous publications in an effort to maximize reliability and validity. The low ICC for these measures suggests that, using this methodology at least, measurement of the amygdala is not reliable. This does not exclude the possibility that less easily identifiable differences in amygdala volume might exist,

but remain beyond our ability to detect with this methodology. The reliability of our measures of caudate volume, callosal area, and WBI is comparable to that reported by Peterson et al. They found an ICC of 0.87 for the caudate and 0.99 for the WBI. However, they calculated the ICC on a random sample of 10 subjects, including 5 normal controls. We calculated ours on the entire sample of 17 patients, which would tend to maximize our detection of inter-rater error.

The finding of an increased cross sectional area of the CC suggests abnormalities of cerebral lateralization. We also found a loss of the correlation between CC area and brain size in our patient group. Differences in CC size in relation to sex and handedness remain unclear. Witelson[30] describes a postmortem study of the CC in 50 brains. Subjects with nonconsistent right hand preference (NCRH) (determined using the same 12-item handedness test,[24] but adapting it, so that subjects were asked to perform the tasks rather than complete a questionnaire) had larger overall callosal area with the greatest difference being seen posteriorly, especially in the isthmus. Significant sex differences also were evident in callosal anatomy. Handedness, as a factor, interacted with sex. The posterior difference due to handedness was seen in males but not in females. This is consistent in general with the hypothesis of females' having less clear lateralization than males. Callosal area correlated with brain weight ($\rho = 0.48$) in that study. Not all authors have found these patterns, however.[31] Increased size in the CC has also been found in epilepsy[32] and in schizophrenia.[33-35] Summarizing 12 published studies from either postmortem (8) or MRI data (4), and 3 further abstracts, Witelson[30] determined that the majority of studies did not show differences in callosal cross-sectional attributable to sex alone, although the interaction with handedness was found as already described.

It is also controversial whether there may be neuronal loss in the brain with aging. Again Witelson[21] found a decrease in CC size with age in normal men, but not in women. Our finding of an inverse correlation between age and CC area in our normal controls (regardless of sex), which was absent in the GTS patients, suggests that other factors related to asymmetry may be more important than age in determining CC area in this group.

Our findings are consistent with the report by Baumgardner et al.[22] of increased CC area independent of age, handedness, intracranial area, or comorbid attention-deficit-hyperactivity disorder. That study considered children and adolescents, whereas the study by Peterson et al.[23] was of adults. Our study suggests that the age difference does not account for the contradictory findings of Peterson et al. Our results also suggest a decline in callosal cross-sectional area with age in normal controls, absent in the GTS patients. This is again consistent with the findings of Baumgardner et al., although they found no correlation with age in any group. Again, this raises the possibility that the "natural history" of CC development in subjects with GTS differs from normal controls; we agree with Baumgardner et al. that assessment of CC size longitudinally is necessary to unravel the interaction between age and callosal morphology in GTS.

It is widely accepted that genetic factors play a part in the development of GTS.[4] Nongenetic factors are likely to include prenatal factors. The predominance of the disorder in males suggests a role for androgenic steroids acting on brain development prenatally.[36] Kurlan[37] has hypothesized that the defective gene in GTS may influence the development and organization of neural networks (including the basal ganglia) under sex hormone control. The loss of cerebral asymmetry seen in our and other studies of GTS implicate some prenatal effect on the developing CNS and is thus consistent with this hypothesis. However, these abnormalities of asymmetry in the CN and increased callosal cross-sectional area in GTS when compared with normal controls may merely reflect neurodevelopmental abnormality. Further abnormalities of asymmetry may subsequently be found in other areas of the brain less accessible to volumetric methods using MRI. Although these findings are important in advancing our understanding of the neurology of GTS, it would be surprising if such generalized abnormalities manifested *only* as GTS. To establish their specificity for GTS, further studies comparing patients with GTS with patients with other neurodevelopmental abnormalities are needed.

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Editorial

Gilles de la Tourette's syndrome

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The Gilles de la Tourette's syndrome (GTS) is a movement disorder characterised by both multiple motor and one or more vocal tics which usually occur many times a day, in bouts. The anatomical location, frequency, complexity and severity of the tics change over time, and characteristically the onset of the disorder is before the age of 21 years (Robertson 1989).

The exact prevalence of GTS is unknown, but a currently accepted figure is 0.5 per thousand (approximately 110000 patients in the USA and 27500 in the UK (Bruun 1984), but even this may prove to be an underestimate. Recent studies (Kurlan et al. 1987; Caine et al. 1988; Robertson and Gourdie 1990) suggest that many cases of GTS are mild, do not come to medical attention and do not require pharmacological treatment.

GTS is found in all cultures and racial groups, but it is rare among the American black population. Most large cohorts of patients with GTS have come from the USA, but substantial numbers of patients have also been reported from the Soviet Union, UK, Japan, FRG, the Netherlands, Denmark and China. These studies plus case reports from all parts of the world highlight the worldwide distribution of the disorder. GTS occurs three or four times more commonly in men than in women, and it is found in all social classes (Robertson 1989).

The clinical characteristics of patients with GTS appear to be independent of culture, as they occur with some degree of uniformity irrespective of the country of origin. The age of onset of symptoms ranges from 2 to 15 years with a mean of 7 years being commonly reported. The most frequent initial symptoms are tics involving the eyes (such as eye blinking), head nodding and facial grimacing, which are also the most common tics. GTS is often referred to as a tic disorder, but patients with GTS usually exhibit a wide variety of complicated movements including touching, hitting, jumping, smelling of the hands or objects, spitting, kicking, stamping, squatting and a range of complexities of gait (Robertson 1989).

The onset of vocalisations is usually later than that of the motor tics, with a mean age of 11 years, and grunting, coughing, throatclearing, barking, snorting, explosive utterances, screaming, humming, hissing, clicking, colloquial emotional exclamations and inarticulate sounds are the usual utterances. Coprolalia (the involuntary inappropriate uttering of obscenities) is reported in approximately one-third of patients and usually has a mean age of onset of 14 years. Copropraxia (the involuntary and inappropriate making of obscene gestures) is reported in 3%–21% of GTS patients. Echolalia (the imitation of sounds or words) and echopraxia (imitation of actions) occur in 11%–44% of patients. Tics and vocalisations are characteristically aggravated by anxiety, stress, boredom, fatigue and excitement, while sleep, alcohol, orgasm, fever, relaxation or concentration leads to a temporary disappearance of symptoms (Robertson 1989).

Many types of behaviour have been reported to occur frequently in patients with GTS. Some types of behaviour, such as obsessive – compulsive disorder, are intimately linked to GTS (see below) and are thus probably an integral part of the syndrome, whereas others, such as hyperactivity, attention deficit disorder and learning difficulties, occur in a substantial number of patients (30%–60%) and are probably often the symptoms for which the patient is referred to the physician (Robertson 1989). Anti-social behaviour, inappropriate sexual activity, exhibitionism, aggressive behaviour, discipline problems (Robertson 1989) and self-injury (Robertson et al. 1989) are found in a substantial percentage of clinic GTS populations. From my experience in clinical (Robertson et al. 1988) and family/pedigree (Robertson and Gourdie 1990) settings and from the data of epidemiological surveys (Caine et al. 1988), it would seem that relatively few GTS subjects in the community exhibit antisocial behaviour and that the reporting of such behaviour may well reflect an artefact of referral, and thus represent ascertainment bias (Robertson 1989).

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Electroencephalogram (EEG) abnormalities have been found in 12%–37% of patient cohorts: abnormalities are non-specific, and there is no evidence of any paroxysmal activity time-locked to the tics (Robertson 1989). Complementing the wide spectrum of clinical manifestations of GTS is an equally wide variety of EEG patterns (Robertson 1989). Visual and sensory evoked potentials have been studied in patients with GTS, but no consistent abnormalities have been demonstrated (Robertson 1989).

Robertson et al. (1988) documented that 71 of 73 patients with GTS have normal CT scans (both abnormalities were septum pellucidum cavities). Other studies have noted CT scan abnormalities in 16 GTS patients; thus, only 18 of 172 documented CT scans have been abnormal, and the abnormalities do not appear to be of direct aetiological significance (Robertson 1989). The 99 normal scans suggest that the pathology in GTS is not structural.

Only one group has used brain imaging studies to investigate function in GTS (Chase et al. 1984, 1986). In the first study a PET (ECAT 11 scanner) scan (using fluorodeoxyglucose F18) showed abnormalities in 5 GTS patients compared with controls (Chase et al. 1984). In the GTS patients there was a relatively close positive association between metabolism in the basal ganglia (particularly the corpus striatum) and metabolism throughout the cerebral cortex. In addition, the cortical distribution of regions in which glucose metabolism seemed to have a close inverse association with the severity of vocal tics clustered in the middle and inferior parts of the frontal lobes bilaterally, extending posteriorly from the frontal poles to the post-central gyrus. Coprolalia, in contrast, was inversely correlated with hypometabolism in the left parasylvian region (Chase et al. 1984). Continuing their work in this area, Chase et al. (1986) assessed 12 untreated GTS patients with matched normal controls using the improved NINCDS neuro PET scanner with higher resolution and sensitivity than the ECAT 11 scanner: at horizontal levels from 8.4 to 8.8 cm caudal to the vertex, non-normalised glucose utilisation rates were approximately 15% below control values in the region of the frontal cingulate and possibly the insular cortex and the inferior corpus striatum ($P < 0.01$).

Chase et al. (1986) also evaluated two men (21 and 40 years old) and one woman (28 years old) with GTS using magnetic resonance imaging (MLI) with a 0.5-Tesla Picker International scanner or a 1.5-Tesla General Electric Sigma scanner, and no definite abnormalities were detected.

Post-mortem studies have not revealed any consistent abnormalities (Dewulf and Van Bogaert 1941; Balthasar 1957; Haber et al. 1986). Robertson et al. (1987) reported that 10 of 80 GTS patients had an abnormally low serum copper level. Two of the 10 were investigated in detail with copper radioisotope studies, and both exhibited abnormalities of copper handling.

The aetiology of GTS is unknown, but it has been suggested that it is inherited as an autosomal dominant with incomplete penetrance (Robertson 1989; Robertson and Gourdie 1990). The biochemical aetiology is also unknown, but it is thought that dopamine is particularly implicated, as dopamine antagonists are useful in the treatment of symptoms in the majority of patients with GTS. In addition, the pathophysiology of GTS may be localised to the basal ganglia (Robertson 1989).

There is a growing amount of literature on the relationship between obsessional disorder and GTS, and it is becoming increasingly evident that there is a clear and strong association between the two disorders (Robertson 1989). To date, 11 studies have documented GTS patients to have obsessional symptoms, traits or illness, varying from 11% to 80% of the patient population (Robertson 1989). Moreover, in controlled studies (Frankel et al. 1986; Green and Pitman 1986; Comings and Comings 1987; Van de Wetering et al. 1988) GTS patients were found to have higher obsessive-compulsive inventory scores and/or behaviours – more than normal control populations and equal to patients with obsessive-compulsive disorder (OCD). Robertson et al. (1988) found that 37% of clinic GTS patients have obsessive-compulsive behaviours and, using standardised rating scales, found much higher scores in GTS patients compared with normative data. In addition, coprolalia and echophenomena were significantly associated with obsessive-compulsive phenomena (Robertson et al. 1988). The argument for a strong association between GTS and obsessive disorder also comes from both pedigree (Pauls et al. 1986; Robertson and Gourdie 1990) and epidemiological (Caine et al. 1988) studies which suggest that not only do patients with mild GTS have significant obsessive-compulsive behaviours but also that OCD may well be a phenotype of the anticipated GTS gene.

Serotonin is the neurotransmitter most implicated in the pathogenesis of OCD (Rapoport 1988). Non-specific neuroradiological abnormalities have been documented in OCD (Behar et al. 1984; Garber et al. 1989). More specifically, it has been suggested (Laplane et al. 1981, 1982; Swedo et al. 1989) that OCD, at least in some patients, may be due to basal ganglia dysfunction, while other findings support evidence of involvement of the caudate nucleus (Luxenberg et al. 1988). One study documented that in OCD metabolic rates were significantly increased bilaterally in the caudate nucleus and hypermetabolism in the orbito-frontal cortex (Baxter et al. 1987).

Single photon emission tomography (SPET) studies using dopamine receptor ligands would be useful to explore the functional aetiology of GTS. They would, for example, enable the hypothesis that dopamine is involved in the GTS to be tested. This clearly has widespread implications.

SPET may clarify whether GTS can be divided into two groups: GTS patients with or without dopamine receptor abnormalities, and in particular whether pa-

tients in the OCD group have dopamine abnormalities. It is likely that serotonin ligands for SPET studies will be available in the near future: this would clearly also have potential to elucidate the role of serotonin in GTS with or without OCD.

Treatment may well be different for the different groups. Dopamine antagonists (haloperidol, pimozide, sulpiride) may be the first choice for those with excessive dopamine uptake, whereas serotonin re-uptake inhibitors (chlomipramine, fluvoxamine, fluoxetine) may be more useful for those subjects without dopamine receptor abnormalities and/or those with more obsessive-compulsive features.

It is suggested that studies employing SPET may therefore be useful in exploring the functional aetiology and treatment of GTS.

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Neuroimaging in child neuropsychiatric disorders

Springer

1. Structural neuroimaging in Gilles de la Tourette's syndrome

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Gilles de la Tourette's syndrome (GTS) is a genetically determined movement disorder characterised by multiple motor and one or more vocal tics (World Health Organisation, 1992; American Psychiatric Association, 1994; Robertson, 1994). Associated behaviour and psychopathology include coprolalia (inappropriate uttering of obscenities) in approximately 30% of clinic patients, echophenomena (copying behaviour) in roughly 40% (Robertson, 1989; 1994) and attention deficit hyperactivity disorder (ADHD) in 20%-90% of clinic patients (Robertson and Eapen, (1992). The most specific psychopathology, which is probably also genetically related, is obsessive compulsive disorder (OCD) (Robertson, 1995). Although GTS is genetically-determined, and the Most likely inheritance pattern is autosomal dominant transmission (Eapen et al., 1993), environmental influences are also important. Thus, prenatal events including maternal illness, eg., severe nausea and/or vomiting during the first trimester and maternal life stress during pregnancy, as well as the sex of the child, may lead to changes in the sensitivity of some dopaminergic receptors, and this could partially determine the eventual severity of expression of the syndrome (Leckman et al., 1990).

The precise brain pathology is, as yet, unknown, in part due the lack of adequate post-mortem (PM) studies. However, in general, PM studies have implicated the basal ganglia. The first PM undertaken on a patient with "Ilidiopathic" GTS was normal (Dewulf and van Bogaert (1941). Subsequent studies, however, have implicated the striatum (Balthasar, 1957; Singer et al., 1991) and the globus pallidus (Haber et al., 1986; Haber and Wolfer, 1992).

Structural neuroimaging allows us to examine anatomy in vivo and provides evidence complementary to these PM studies. Functional neuroimaging, examining regional blood flow, metabolism and receptor function, provides another avenue of exploring the neuropathological lesions of GTS. In this chapter, I confine myself to a description of structural neuroimaging studies, including computed tomography (CT) and magnetic resonance imaging (MRI).

In order to gain as comprehensive review of the literature as possible, MEDLINE and PSYCHLIT searches were performed using the titles "Tourettes", X RAY, CT scan and MRI scan. No articles involving GTS and X ray examinations were found. The literature on CT and MRI scans in patients with GTS will be reviewed. When considered relevant,, in some cases, more details will be given about individuel cases or the study, to illustrate the areas possibly involved in the pathogenesis of GTS.

Individual CT case reports

The first report of CT scan in GTS was by Yeragani et al (1983), who documented a 12-year-old girl with GTS, whose CT showed mild increase in the density of the caudate nuclei, thought to be due to calcification. There was no relevant family history. The patient had elevated levels of beta-phenylethylamine and the catecholamine metabolites normetanephrine, MHPG and 3-methoxytyramine, and a decrease in urinary 5-HT.

Soon afterwards, Shaenboen et al. (1984) reported the CT appearances in a 16 year old girl with GTS who had a brief acyanotic spell at 30 h of age, and delayed speech, but an otherwise unremarkable history. CT showed markedly enlarged occipital horns

of both lateral ventricles. Lakke and Wilmink (1985) reported a 27 year old man with GTS whose CT showed a pineal tumour and calcification in the region of the third ventricle and periaqueductal grey matter. Kjaer et al. (1986) described the CT of a 28 year old woman with GTS, which showed a large porencephalic cyst in the right hemisphere, virtually replacing the temporal lobe and involving the basal ganglia, and contrast enhancement in the region of the left basal ganglia. The patient had been born 4 weeks premature, had a left sided clonic seizure at weeks old and a hyperactive stretch reflex on the left.

Vieregge (1987) documented monozygotic (MZ) twins concordant for GTS. Both had unremarkable gestations and births. In preschool age both were hyperactive and "destructive"; developmental milestones were normal. From the age of 6 years both showed compulsive behaviour. Motor tics began at the age of 12 (B) and 13 (A) years, followed soon after by vocal tics. The more severely affected twin (B) showed marked asymmetrical left involvement and soft neurological signs. There was a positive family history of GTS. Neurophysiological investigations (eg., visual evoked responses) were normal, as was the EEG, apart from muscle artefacts. CT revealed some ventricular asymmetry in both, without observable differences.

An interesting, acquired Tourette-like syndrome was reported by Northam and Singer (1991) in a 6 year old girl following presumed herpes encephalitis. She had no prior neuropsychiatric history and no family history of tics or OCD. After a 3 day prodrome of headaches, malaise, nausea and vomiting, she developed a fever (39.5.c) and presented, with focal motor seizures involving the left side of the face and left arm. CT without contrast medium was normal. She was treated with diazepam and phenytoin. Repeat CT on hospital day 3 showed an acute haemorrhagic lesion in the right mesial temporal region, and MRI on day 5 a small high-signal lesion in this region, involving both grey and white matter, and progressive oedema in the right temporal lobe, basal ganglia and thalamus.

After a presumptive diagnosis of herpes encephalitis, the patient was treated with acyclovir and maintained on phenytoin. At discharge 2 weeks later she had obvious neurological signs (eg., mild left facial and arm paresis), marked anxiety, personality change, emotional lability and hyperactivity. Two weeks later she developed both motor and vocal tics. CT two months after the onset showed encephaloclastic change in the right temporal lobe. Four months later she had no neurological abnormalities apart from the tics, which responded to small doses of pimozide.

Of course, individual case studies, while useful, are limited by difficulty in knowing the relevance the pathological findings in specific individuals with GTS, when most do not have any abnormalities. Thus, studies of series of patients with GTS allow us to determine whether any more systematic abnormalities are to be found.

CT studies in patients with GTS

Caparulo et al. (1981) reported CT abnormalities in (6) 38% of 16 patients with GTS, including mildly enlarged lateral ventricles (in 2 cases), substantially enlarged, symmetrical or asymmetrical ventricles (in 3) and an enlarged right Sylvian fissure. No systematic relationship was found between EEG and CT scan abnormalities. No mention was made of family history.

In a controlled study (Harcherik et al. 1985) CT was performed on 19 patients with GTS and compared with studies of patients with infantile autism, ADHD, and a language disorder, and a control group of medical patients (with headaches, post concussion or inner ear problems) . No significant difference was found between groups or controls with respect to total ventricular volume, right/left ventricular volume ratio, ventricular asymmetry, ventricle/brain ratios or brain density.

Chase et al. (1986) reported seven normal CT studies in a cohort of nine patients with GTS. The only abnormalities were mild ventricular dilatation in a 21 year old man, and mild, diffuse cortical atrophy in a 40 year old man.

Regeur et al. (1986) reported 47 normal CT examinations in a cohort of 53 patients with GTS. Abnormalities included a small occipital arachnoid cyst, a suprasellar epidermoid, a large defect in the right temporo parietal region, slight cortical atrophy, and asymmetry of the ventricles in two cases.

The largest CT study is that of Robertson et al. (1988) who found 71 of 73 patients to have normal CT examinations. The only abnormalities were a cavum of the septum pellucidum and two patients, both of whom had a history of head banging. The report of normal CT studies by Lees et al. (1984) included the same patients.

Thus, although some groups have found subtle abnormalities of ventricular size or symmetry, and there are occasional reports of cerebral anatomical abnormalities or structural pathology, the overall conclusion must be that there are no structural abnormalities are systematically revealed by CT in patients with GTS. The literature reviewed, reveals that 154/177 (87%) of the CT studies were normal. The mutation abnormalities described, may not distinguish GTS from other neuropsychiatric disorders presenting in childhood. It is of course possible that

more subtle structural abnormalities are not detectable with CT.

MRI case reports in patients with GTS

To date, there have been five reports of normal MRI. Chase et al. (1986) reported two men aged 21 and 40 years, and a woman of 28 years who had normal MRI. Robertson and Trimble (1991) reported a 21 year old woman with typical symptoms with no associated behaviours, whose MRI was also normal, as were the neurological examination and EEG. Relevant family history was that a paternal aunt had facial tics. Hartman and Yuvarajan (1994) described an 82 year old woman who presented with motor and vocal tics, and coprolalia, which had developed in the months following an acute confusional state associated with posterior neck pain 10 years earlier, presumed to be encephalitis lethargica; the patient would therefore not fulfil criteria for "Ilidiopathic" GTS both because of her age and the organic insult. Nevertheless, despite her age and "Ilorganic" insult, MRI was normal.

Two case reports have documented MRI abnormalities. Sandyk (1988) reported a 7 year old boy who also had coprolalia, abnormal sexual behaviour and ADHD. At the age of 5 years he was prescribed methylphenidate (ritalin) for his ADHD. Neurological examination was normal. MRI being showed asymmetry of the cerebral peduncles, the left being larger than the right.

Robertson et al. (1990) documented a 19 year old man with severe GTS, obsessive compulsive (OCB) and self-injurious behaviours (SIB) and coprolalia, whose symptoms were so severe that he required psychosurgery, in the form limbic leukotomy. He was born at term by caesarian section following an antepartum haemorrhage due to placenta praevia; he was nursed in an incubator for the first four days of his life. His presurgical MRI revealed a single high signal focus in the right globus pallidus; CT and EEG were both normal.

MRI studies in GTS

There have been several MRI studies in GTS patients, although some have not been on patients with "pure" GTS and have included patients with, for example, GTS plus ADHD and Asperger's syndrome (AS). Nevertheless all studies will be reviewed and conclusions drawn where possible.

A preliminary MRI study of 10 children with GTS who also had ADHD found significantly larger ventricles (6.0% vs 3.2% of total brain volume; p=0.007), larger caudate nuclei (2.7% vs 2.5% of total brain volume; p=0.05) and trends toward a smaller globus pallidus compared with normal controls (Denkla et al., 1991).

Demeter (1992) compared 10 adult patients with 10 medical controls using MRI: no quantitative differences were found. However, two of the patients with GTS had focal abnormalities involving the basal ganglia, one of whom had a family history of tics.

Berthier et al. (1993) investigated nine males with GTS, and compared them to seven with concurrent GTS and AS using MRI. Eight of the patients with GTS had normal MRI; the only one patient with abnormal MRI had moderate enlargement of lateral ventricles. In contrast, five of the seven (71%) with both GTS and AS had abnormal imaging. Peterson et al. (1993) used MRI to examine 14 caucasien patients with GTS aged 18-49 years (11 men, 3 women) who had minimal lifetime neuroleptic exposure (10 had never received neuroleptics), and compared them with age- and sex- matched controls, who had no personal or family history of tic or obsessive compulsive symptoms. All patients were medication free for a month prior to imaging. Results showed a significant reduction in the volume of the left (but not the right) lentiform nucleus. Post hoc analyses revealed smaller mean volumes of the caudate and lentiform nuclei and globus pallidus then in controls on both the right and the left. Further analyses of basal ganglia asymmetry indices suggested that in GTS the basal ganglia do not have the volumetric asymmetry (left greater than right) observed in normal controls. In contrast, the patients had lateral ventricular asymmetry not seen in the normal subjects, which appeared to be due to a 33% increase in mean volume of the right lateral ventricle over control values, compared with a 12% increase the left lateral ventricle. Interestingly, vocal but not motor tic severity correlated positively with all basal ganglia volumes (globus pallidus excepted), on both right and left sides.

Singer et al. (1993) performed an MRI study on 37 outpatient children with GTS (29 boys, 8 girls; age range 7-16, mean 11.5 years) and compared them with 18 controls (14 boys, 4 girls; age range 6-15, mean 9.8 years). Of the patient 78% had a family history of either tics or OCD; of the controls none had a family history of tics or GTS. At the time of MRI 25 patients (68%) were receiving medication, most commonly desipramine (in 14); 6 were receiving neuroleptics. There were no statistically significant differences in the size of the right or left caudate nucleus, putamen, globus pallidus or lateral ventricles between the groups. In contrast, there were significant differences in symmetry in the putamen and lentiform nucleus. Virtually all the controls had a left-sided predominance of the putamen, whereas in 13/37 patients (35%), a right predominance exceeded

that of any control. Statistical comparisons of 18 patients with and 19 without ADHD and the controls showed significant differences in volume of the left globus pallidus and lenticuform nucleus asymmetry. In the GTS+ADHD group, the left globus pallidus was significantly smaller than the right, and the lenticuform asymmetry was due to a greater right-sided predominance in the GTS+ADHD group.

Peterson et al. (1994) measured the midline cross-sectional area and other morphological features of the corpus callosum (CC) on MRI of 14 medication free patients with GTS (11 men; 3 women) and 14 normal controls (matched for age, sex, socio economic status and handedness; all were right handed); the ages ranged between 18 and 49 years. Exclusion criteria in the patient group included history of head trauma causing loss of consciousness, past or current depressive or psychotic illness, neurologic illness, pregnancy and a history of alcohol or drug abuse. No control subject gave any reported no personal or family history of tics or obsessive-compulsive symptoms. The cross-sectional area of the CC correlated positively with brain size and basal ganglia volumes. Overall, CC area was 17.7% less in the patients than in the controls ($p < 0.006$); there was also a 10% reduction in the circumference of the CC. The reduction in area was similar in degree throughout the CC. Analyses of covariance with total midsagittal area of the head revealed the reductions in the overall CC area of the CT and all its subsections to be statistically significant. The width of the CC tended to be insignificantly less throughout (5%-11%); its overall length from rostrum to splenium was significantly reduced (by 5.3%). The bending angle and mean curvature of the CC were both increased in GTS, suggesting that the CC was less rounded than that of normal controls. Worst-ever motor tic symptoms showed the strongest significant correlation with the length of the CC in the patients. These findings suggested that structural interhemispheric connectivity may be aberrant in the central nervous system in GTS, and the authors suggested that they provide indirect supportive evidence for the presence of altered cerebral lateralisation in GTS.

A careful monozygotic (MZ) twin study was undertaken by Hyde et al. (1995) who performed morphometric analyses of MRIs of 10 MZ twin pairs concordant for tic disorders, but discordant for tic severity. Twins were considered to be MZ only if their blood samples matched on all 19 blood antigens tested. The mean age of the group was 16.3 years (range 9-31). No focal neurologie abnormalities were found other than tics, and no patient had abused drugs. The right caudate nucleus volume was slightly (6%) but significantly reduced in the relatively more severely affected twins compared with the less affected ($t=3.34$; $p < 0.009$). Most of the difference was attributable to reduced volume of the anterior right caudate nucleus ($t=2.26$; $p < 0.02$), which was smaller in the more severely affected twin in nine out of ten sets. There was also a trend for the left anterior caudate nucleus to be smaller in the more severely affected twin sets. In addition, the mean volume of the left lateral ventricle was 16% less in the more severely affected than in the less severely affected twins ($t=2.86$; $p < 0.01$). In eight of ten sets, the more severely affected twin had a smaller left lateral ventricle. The normal left greater than right asymmetry of the lateral ventricles was not present in the more severely affected twins ($t=-3.39$; $p < 0.008$). In addition, the difference within a pair in the degree of loss of the normal ventricular asymmetry correlated with the difference within a pair in the severity of the tic disorder. In contrast to the maintenance of the normal ventricular asymmetry in the less severely affected twins, both less and more severely affected twins had a predominance of the caudate nuclei and striatum as a whole. The authors suggested that because MZ twins are genetically identical, the structural abnormalities must reflect adverse environmental events.

The nost recent study is from our group (Moriarty et al. submitted for publication). In brief, MRI was used to assess 17 patients with GTS and eight normal controls. There were no differences in volume between patients and controls in the amygdala or caudate nuclei and no difference in whole brain index. There was, however, loss of the normal caudate asymmetry in the patients. The area of the corpus callosum was significantly greater in patients than in controls and there was a loss of the normal correlation between it and whole brain index.

In summary, and not unexpectedly, the MRI studies reveal substantially more abnormalities than the CT investigations. These include primarily those in the basal ganglia and lateral ventricles: abnormalities of caudate nucleus size (mostly decrease) and loss of normal caudate nucleus asymmetry: in the asymmetry of other basal ganglia (eg., putamen and lentiform nucleus); and in the size of the lateral ventricles, with loss of normal ventricular asymnetry. Finally, two studies have found abnormalities of the corpus callosum, likely to reflect abnormalities in the development of cerebral lateralisation (Witelson 1993). However, one group found an increased cross-sectional area of the corpus callosum in GTS patients, while the other found it lobe reduced, reinforcing the fact that structural neuroimaging studies have methodological limitations, and further systematic investigation will be needed to clarify these findings.

Conclusions

While PM and earlier CT studies did not show convincing or consistent abnormalities in patients with GTS, recent studies with the more sophisticated techniques of MRI show a reasonable consistency in abnormalities of cerebral lateralisation, affecting particularly the basal ganglia. Whether or not these structural abnormalities are genetically determined, or, as suggested by Hyde et al. (1995), are the result of environmental factors is as yet unknown. More controlled studies and more twin studies are called for. The next phase of neuroimaging investigations in GTS be functional MRI studies, which might bridge the gap between the traditional functional studies such as single-photon emission tomography and positron emission tomography (PET) and traditional MRI, and allow further insights into the pathophysiology of GTS.

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Abnormalities of Copper in Gilles de la Tourette Syndrome

Mary Robertson, Kenneth Evans, Anthony Robinson, Michael Trimble, and Peter Lascelles

The Gilles de la Tourette syndrome is a disorder whose etiology and pathogenesis are little understood. The number of biochemical abnormalities described in this disorder is minimal. Ten of a total of 80 patients were found to have an abnormally low serum copper. A report is presented on two patients who consented to further detailed investigation and in whom copper radioisotope studies were carried out. Both exhibited abnormalities of copper handling, in that we observed an abnormally fast disappearance of copper from the plasma and an abnormally slow uptake by the liver. The rates of intestinal absorption and urinary excretion were normal. We did not identify an abnormal site of sequestration of the metal in the body.

Introduction

Prompted by reports of copper abnormalities in patients with movement disorders such as Wilson's disease (Cumings 1948), Menkes's disease (Menkes et al. 1962), Huntington's chorea (Perry 1961), and at least four other rare conditions (Willvonseder et al. 1973; Godwin-Austen et al. 1978; Haas et al. 1978; Parker 1985), we decided to request assays of serum copper and ceruloplasmin in patients with the Gilles de la Tourette syndrome (TS) and found that a significant proportion seemed to have abnormal results. Thus, the finding of 10 of 80 patients with abnormally low levels in this specialist population, with symptoms, made it unlikely that this could be attributed to a normal distribution curve.

Eighty subjects who were seen and examined by one of us (M.R.) over 4 years (1980–1984) and who fulfilled the diagnostic criteria for the Gilles de la Tourette syndrome (TS) laid down in the *Diagnostic and Statistical Manual of Mental Disorders* (1980) were investigated. All were British, and all were white. The clinical features and neurological examinations of the first 53 subjects have been described elsewhere (Lees et al. 1984). Wilson's disease was excluded in all 80 subjects on the basis of detailed clinical examination. As abnormalities of copper have never been described before in TS, it was decided to further investigate two patients in detail, using radioisotope studies. The results were compared with similar information collected by one of us (A.R.) over many years

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in 6 normal controls, 14 subjects heterozygous for Wilson's disease, 7 untreated subjects homozygous for Wilson's disease, and 7 treated subjects homozygous for Wilson's disease.

Case Reports

Subject 1

Subject 1 was born at term to unrelated parents after a normal pregnancy and delivery in 1936; he was the sixth of nine children. There is no family history of neurological illness nor movement disorder. One brother was mentally retarded, another treated for a "persecution complex," and a third had a brain tumor; one sister had consulted a psychiatrist. As a child, the subject bit his nails, was prone to temper tantrums if frustrated, and exhibited breath-holding attacks. His childhood was disrupted by World War II and his mother's death when he was 3 years old. Furthermore, there was maltreatment by several older relatives. At school, he was academically good, passed secondary school examinations, but received no formal education after the age of 16 years. After school, he enjoyed a good work record until 1980, when he was made redundant. He was married at the age of 22 years, fathered 3 normal children, and was subsequently divorced.

At the age of 7 years, the subject developed a facial twitch that was soon followed by a wide repertoire of motor and vocal tics. The symptoms wax and wane in severity and affect various parts of the body at different times. He is able to suppress them voluntarily for a short while, but at the expense of experiencing anxiety. The symptoms disappear in sleep, are reduced with alcohol, and worsen with anxiety. He also experiences echolalia, echopraxia, arithmomania, and the uttering of obscenities (coprolalia). As a child, he was thought to have St. Vitus dance.

He presented to the National Hospitals at the age of 44 years and was diagnosed as having TS. There was no other relevant previous medical or psychiatric history. On examination, he answered questions appropriately, but appeared tense and anxious. His talk was interrupted by expletives and inappropriate fluctuations in pitch. There was some depression of mood, with diurnal variation, but there were no other biological features. Cognitive testing was normal, and there was no evidence of psychosis.

Physical examination revealed multiple motor tics and vocalizations, and mild left-sided dysdiadochokinesis. There were no Kayser-Fleischer rings. He was completely right-handed (Annett 1970). Normal laboratory investigations included hemoglobin, full blood count, ESR, platelets, serology (WR, VDRL, TPHA), cholesterol, prolactin, urea and electrolytes, liver function tests and protein electrophoresis, serum vitamin B_{12}, red cell folate, uric acid, T4 and free T4, creatinine, iron, total iron binding capacity, calcium, inorganic phosphorus, alkaline phosphatase, glucose, and 24-hr urinary copper excretion. An autoimmune screen was negative for thyroid and gastric parietal cell antibodies, smooth muscle and mitochondrial antibodies, but was positive at 1 in 20 for antinuclear bodies. Two electroencephalograms were similar in that they were flat and featureless with low voltage; there was frequent generalized and focal muscle activity corresponding to involuntary movements. A computerized axial tomography (CAT) scan was normal, as was the electrocardiagram. The subject was assessed on the Wechsler Adult Intelligence Scale and had verbal IQ of 103, a performance IQ of 103, and a full-scale IQ of 103 (Department of Neuropsychology, National Hospital, Queen Square).

The subject has been admitted to the hospital on several occasions and has been

followed-up monthly as an outpatient. Depending on what symptoms were disturbing him most—tics, anxiety, depression, or insomnia—he was treated with various medication regimens, which have included tranylcypromine, clomipramine, lorazepam, diazepam, chlormethiazole, glutethimide, haloperidol, pimozide, chlorpromazine, fluspirilene, sulpiride, clonidine, phenobarbitone, and carbamazepine. There have been temporary reliefs of mood, anxiety, and movements, but no consistent and fundamental change in his presentation or mental state.

Subject 7

Subject 7 was born in 1962 by caesarian section, for cephalopelvic disproportion, to unrelated parents after a normal full-term pregnancy. He is the second of two children. His mother has grand mal epilepsy and his elder sister has absence seizures. There is no family history of psychiatric illness or movement disorder.

His early development was unremarkable, and he achieved normal milestones. He wet his bed as a child and has always bitten his nails. He was at school until he was 17 years old and was academically average. He enjoyed a good work record as a printer. His psychosexual development was normal, and he married at the age of 22 years.

At the age of 11 years, the subject began to shake his head from side to side. Soon other involuntary movements followed, and at the age of 16 he developed vocalizations in the form of throaty grunts. The symptoms, which wax and wane in severity, can be suppressed voluntarily and disappear in sleep. They are worsened when he is excited or annoyed, and reduced when he plays football, relaxes, or is in company. He was diagnosed as having Sydenham's chorea at the age of 14 and was treated with phenobarbitone for 3 years, to no avail.

He presented at the National Hospitals at the age of 18 years and TS was diagnosed. There was no other relevant previous medical or psychiatric history. On examination, his talk was normal, his mood euthymic, and there were no psychotic phenomena. He exhibited multiple tics of head, shoulders, and trunk and made occasional grunts. Physical examination was unremarkable. No Kayser-Fleischer rings were detected. He was predominantly right-handed (Annett 1970). Normal laboratory investigations included hemoglobin, full blood count, ESR, calcium, inorganic phosphorus, alkaline phosphatase, liver function tests, protein electrophoresis and immunoglobulins, urea, glucose, electrolytes, 24-hr urinary copper excretion, and cytogenetic studies. Electromyogram (EMG) studies and CAT scan were normal. Two electroencephalograms were moderately and diffusely abnormal, with excess slow activity and a rather paroxysmal character, but no frank epileptic activity was seen, and there were no focal features.

He was assessed on the WAIS and had a verbal IQ of 100, a performance IQ of 111, and a full-scale IQ of 105; there was no sign of a specific language problem (Department of Neuropsychology, National Hospital, Queen Square).

A double-blind trial of single-dose clonidine, placebo, and haloperidol was carried out, and in view of his good response to haloperidol, he was discharged on this drug, taking 2 mg daily. He is followed-up regularly in the outpatient department, and 4 years later (aged 22 years), maintains improvement concerning his tics, with a normal mental state and good adjustment to life. His only medication has been haloperidol 2 mg daily.

In view of the abnormalities of copper metabolism, his parents and sister were investigated. None of them had abnormal movements nor a Kayser-Fleischer ring. Blood was taken for estimation of serum copper and ceruloplasmin; the levels of both in his father

were normal, whereas the copper in his mother and sister were identical and just above the upper limit of normal (21.6 μmol/liter). Ceruloplasmin levels were normal.

Methods

^{64}Cu with a specific activity of 5–30 mCi/mg copper, in the form of cupric chloride, was obtained from Amersham International plc (code CMS2) and was used for both intravenous and oral administration. The ^{64}Cu was passed through a Millipore filter (Millipore Corp., Bedford, MA), diluted with saline, and sterilized in an autoclave. The standard and intravenous doses were calculated by weight, and the dose syringes flushed with the patient's blood and checked for residual radioactivity. No significant radiocopper appeared to be sequestered by the plastic syringes, which was confirmed in a separate experiment using nonradioactive cupric chloride of the same concentration as the doses. Two hundred microCuries of ^{64}Cu were given intravenously, and heparinized blood samples were collected after 5, 10, 15, 20, 30, 40, 50, and 60 min and then at 1.25, 1.5, 2, 3, 5, 7, and 10 hr. A further 4 samples were taken up to 48 hr after the injection. All stools and urine were collected. Three days later, 150 μCi of ^{64}Cu was given orally. Twelve blood samples were taken during the next 48 hr. Stools and urine were collected for 3 days. Samples of plasma, stool, and urine were counted together with a standard, made up from the dose solution, and the percentage of the dose in these samples was calculated. The percentage in the plasma after both i.v. injection and oral administration was plotted against time, and the absorption rate of ^{64}Cu from the gut was calculated using a deconvolution technique (Godwin-Austen et al. 1978). The percentages absorbed during 6-min intervals were plotted and the total percentage during 10 hr was calculated.

To measure the ^{64}Cu incorporated into ceruloplasmin, samples were incubated with diethyldithiocarbamate to release ^{64}Cu bound to albumin. Free copper was then absorbed onto activated charcoal, leaving the ceruloplasmin-bound ^{64}Cu in solution (Tauxe et al. 1966). The percentage in the supernatant was again plotted against time on semilog paper to give the ceruloplasmin incorporation curve.

The ratio of the activity over the liver and thigh was obtained using a scintillation probe and rate meter. The ratio was normalized to unity by the technique of Osborn and Walshe (1964). The normalized ratio was obtained during the first 2 hr and again at 24 hr. Ceruloplasmin was measured by the method of Houchin (1958). Copper in plasma and urine was measured by the method of Meret and Henkin (1971).

Results

Table 1 shows the data for all 10 subjects from the total sample of 80 subjects with TS in whom serum copper was abnormal. The differences in ceruloplasmin incorporation between representative normal, heterozygous Wilson's disease, and homozygous Wilson's disease subjects are shown in Figure 1. The plasma disappearance curves are also included. Of 6 normals and 14 heterozygotes for Wilson's disease examined, we have found that there is an overlap between the lower normal and upper heterozygote ranges. In these subjects, there is always a quantifiable percentage uptake of copper. However, of 14 subjects with Wilson's disease, 7 untreated and 7 treated, there is invariably zero incorporation of radiocopper into ceruloplasmin.

The shaded area of Figure 2 represents the range of the ceruloplasmin incorporation curves for the 14 obligate heterozygous Wilson's disease subjects. The dotted curves are

Table 1. Summary of Data on the 10 Reported Subjects with TS Exhibiting Abnormalities of Serum Copper

Subject	Age (years)	Sex	Age at onset	Wechsler[a] full scale	Family history of tics	Neurological examination	EEG	Scan	Serum copper (μmol/liter)[b]	Serum ceruloplasmin (μmol/liter)[b]
1[c]	44	M	7	103	No	ABN[d]	ABN	N	11.0	0.9
2	27	M	7	110	Yes	N	N	N	11.9	1.3
3	18	M	11	102	Yes	ABN	ABN	N	12.2	1.3
4	23	M	5	87	No	ABN	N	N	12.2	1.6
5	16	M	7	75	No	ABN	ABN	N	11.0	1.6
6	17	M	1	107	Yes	N	N	N	10.0	1.1
7[c]	22	M	10	105	No	N	ABN	N	9.3	1.5
8	40	M	3	114	Yes	N	N	N	12.3	1.2
9	22	M	4	87	Yes	N	ABN	—	12.4	1.6
10	14	F	7	—	No	N	—	—	11.4	1.4
Normal	—	—	—	—	—	—	—	—	12.5–19.0	1.3–2.9

[a]WAIS or WISC, as appropriate.

[b]Conversion factor by multiplication: SI → traditional units—serum copper: 6.4 to μg/100 ml, serum ceruloplasmin: 15.0 to μg/100 ml.

[c]Subjects described and investigated in further detail.

[d]N, normal; ABN, abnormal.

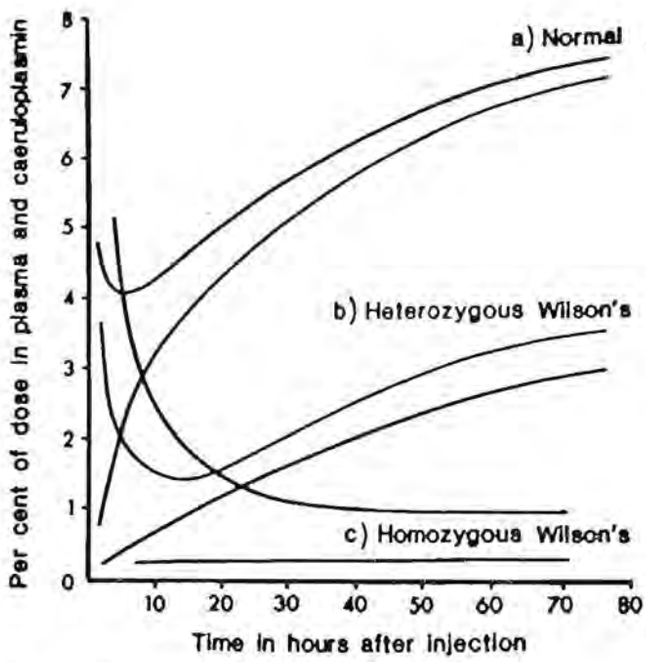


Figure 1. Curves of total plasma radioactivity and incorporation into ceruloplasmin of intravenously administered radioactive copper in three representative subject groups: (A) normals (6), (B) heterozygous Wilson's disease (14), and (C) homozygous Wilson's disease (7 treated, 7 untreated). The upper curve for each subject is that of total plasma activity, and the lower curve is the incorporation of copper into ceruloplasmin.

the corresponding data for the two subjects with TS (heavy line, subject 7, and the light line, subject 1). It can readily be seen that the incorporation of copper into ceruloplasmin of subject 1 is less than the lowest found in subjects heterozygous for Wilson's disease and markedly less than any normal individual. That of subject 7 is also much lower than the lowest normal, but not outside the heterozygote range. The normal range overlaps the upper range for heterozygotes to only a minor degree and is not shown in Figure 2. The continuous lines represent total plasma radioactivity for the two subjects.

The absorption rate curve of subject 1, derived via deconvolution from the curves of percentage ^{64}Cu in the plasma after both intravenous and oral administration, is shown in Figure 3. A similar curve for subject 7 is not available.

The uptake of copper by the liver (Figure 4), as measured by the liver/thigh ratio, was only available for subject 7. Figure 5 shows the rate of disappearance of radioactive copper from the circulation in the two subjects over the first 48 min following injection compared with 6 normals.

Discussion

Abnormalities of copper metabolism have been described in a number of diseases affecting the central nervous system, such as Wilson's disease (Cumings 1948), Menkes's disease (Menkes et al. 1962), Huntington's chorea (Perry 1961), and at least three other rare

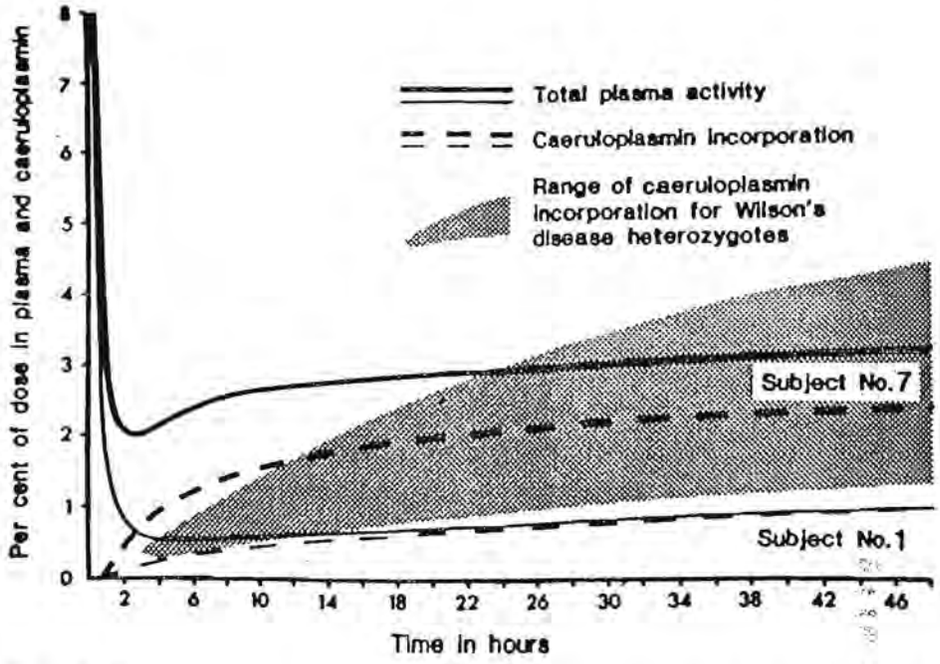


Figure 2. The curves for incorporation of radioactive copper into ceruloplasmin in the two subjects with Gilles de la Tourette syndrome are shown by the heavy and light broken lines, respectively, as compared to the range of 14 subjects heterozygous for Wilson's disease (shaded area). The heavy and light continuous lines indicate total plasma activity for the same two subjects.

conditions (Willvonseder et al. 1973; Godwin-Austen et al. 1978; Haas et al. 1981). On history, examination, and special investigation of our subjects, there was no evidence to suggest that the low copper and/or ceruloplasmin levels found were attributable to any of these conditions.

Recently, Parker (1985) described the syndrome of hereditary whispering dysphonia. The affected individuals belong to an Australian family in which at least 20 members have inherited torsion dystonia, which presents as an unusual speech disorder in that when they try to speak, they exhibit only a faint whisper. What is of special interest is that two siblings with an affected mother have similar clinical manifestations and detailed biochemical investigations, including a liver biopsy in one child and the presence of a Kayser-Fleischer ring in the other, suggested Wilson's disease, yet none of their immediate or distant relatives had any of the biochemical changes found in Wilson's disease, and there was no suggestion of consanguinity in that branch of the family.

Two of our subjects were found to incorporate copper into ceruloplasmin, but at a considerably reduced rate. We have insufficient data to determine whether or not this finding correlates with the serum ceruloplasmin concentration, but in patients homozygous for Wilson's disease it does not, there being almost zero incorporation even when the ceruloplasmin concentration is normal. In Menkes's disease and related conditions, this also seems to be the case (Godwin-Austen et al. 1978; Haas et al. 1981). Therefore, interpretation of this observation in our two subjects is difficult.

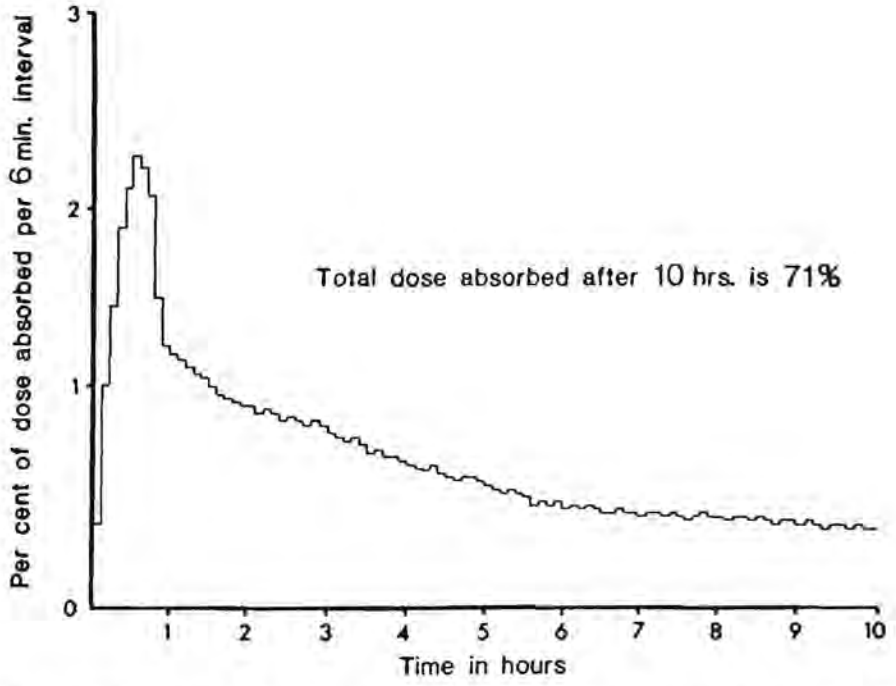
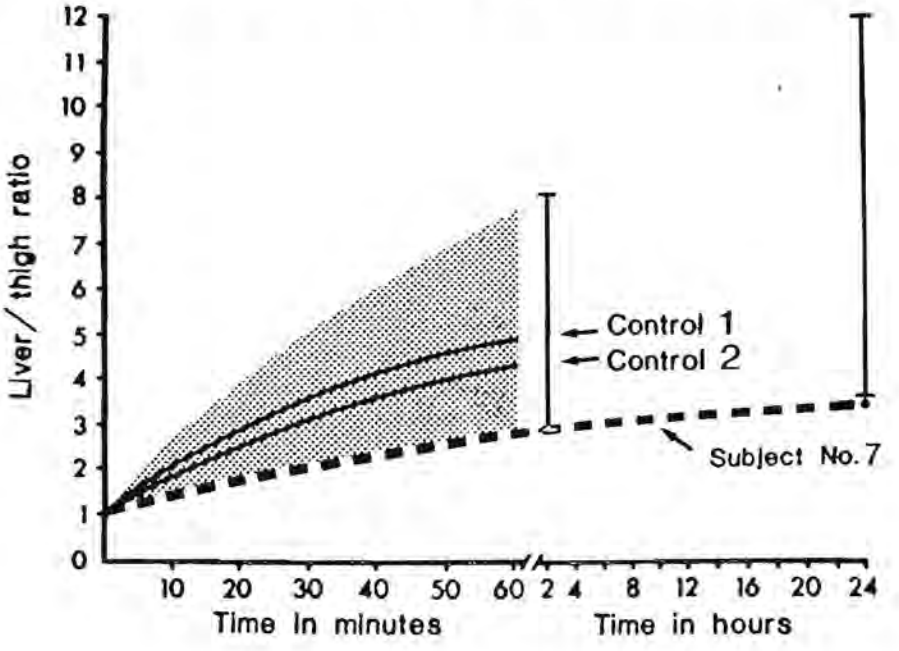


Figure 3. This shows normal gastrointestinal absorption of radioactive copper during 6-min intervals in subject 1 with Gilles de la Tourette syndrome.

Figure 4. Liver/thigh uptake of radioactive copper in two normal individuals and in subject 7. The normal ranges shown are those of Osborn and Walsh (1964).



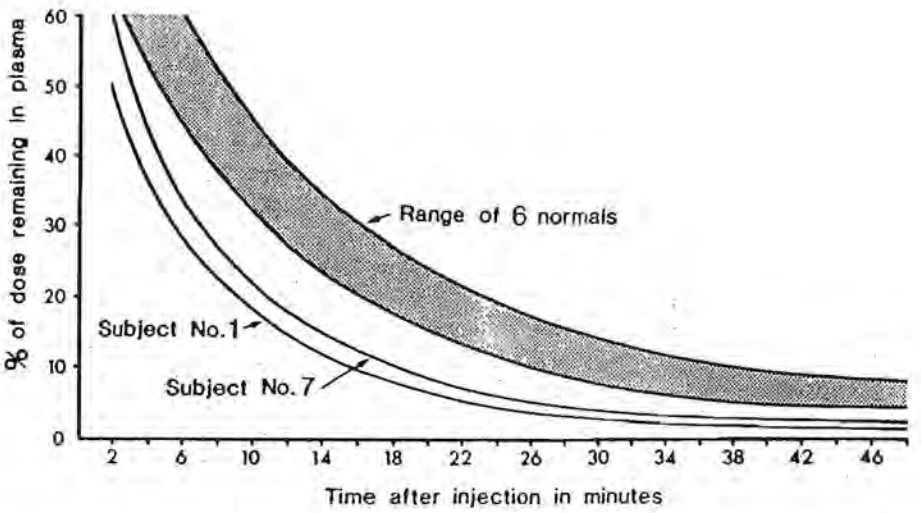


Figure 5. Curves of the disappearance of radiocopper from the circulation in the two subjects and a range from 6 normal individuals.

A further abnormality noted was that the disappearance of radiocopper from the circulation was much greater in our two subjects than in the normal subjects (Figure 5). At 30 min, subject 1 had 3.7% of the dose of injected copper in the plasma compartment, and subject 7 had 4.2% of the dose in the plasma compartment. Our normal range at this time is 7.7%–14.4%. Whether or not this indicates a higher affinity for copper by a protein, such as metallothionein, is clearly not known. In Wilson's disease, this protein is found to be abnormal (Evans et al. 1973); therefore, it cannot be discounted that in other disorders displaying abnormalities of copper metabolism there are also abnormalities of other metal-binding proteins. The hepatic uptake of copper as measured by the liver/thigh ratio was also abnormal (Figure 4). At 15 min, the ratio was 1.4, which very closely approaches the value of 1.5 obtained by Osborn and Walshe (1967) for their group of asymptomatic subjects homozygous for Wilson's disease. At this stage of the disease, the liver is in a phase of copper accumulation, with many binding sites saturated, but as information from liver biopsies in our subjects was not available, it has not been possible to measure the concentration of liver copper in these subjects with TS.

The intestinal absorption rate of copper, as calculated from the oral and intravenous radiocopper data in subject 1, is normal; therefore, neither delayed absorption in the upper gastrointestinal tract, as in Menkes's disease, nor binding in the distal part of the gastrointestinal tract, as in the patient reported by Godwin-Austen et al. (1978), can be implicated in the pathogenesis of the decreased serum copper levels.

Total serum copper is accounted for by a protein-bound fraction tightly bound to ceruloplasmin and a small fraction loosely bound to amino acids (Neumann et al. 1967). Both fractions are involved in the transport of copper in blood to the liver where, in rats, the copper is incorporated into a protein of low molecular weight of about 10,000 (Marceau and Aspin 1973). Other animal studies suggest that this might be metallothionein (Brenner et al. 1976; Hartman and Weser 1977). In Wilson's disease, this protein has been found to have a copper binding constant 4 times that of normal (Evans et al. 1973) and may be responsible for a large pool of copper that makes copper unavailable to the normal

pathways of copper metabolism. This, in part, may be responsible for the low concentrations of serum ceruloplasmin found in this condition.

As our patients do not have Wilson's disease, it is unlikely that an identical metabolic disorder exists, but our findings do suggest that there is a disturbance of copper metabolism in a subgroup of patients with TS. The biochemical changes were not associated with any particular motor, psychopathological, or demographic variables. It is important to note that there was no association with current or previous medication, including butyrophenones, benzodiazepines, tricyclic antidepressants, and anticonvulsants. The fact that only an apparent subgroup of patients with TS exhibits an abnormality of copper metabolism highlights again the heterogeneity of the syndrome previously reported by ourselves (Lees et al. 1984) and other investigators (Shapiro et al. 1978; Comings and Comings 1985).

It is felt that these abnormalities do not carry implications for any new form of management. Indeed, copper treatment was not attempted at this stage because of the dangers associated with possible abnormal distribution in various body tissues that may not all be copper deficient. In addition, administration of copper to children with Menkes's disease has not been shown to be effective.

We do not necessarily postulate that the two abnormalities, i.e., TS and abnormal copper metabolism, are causally related. However, we suggest that a genetic mechanism may be responsible, as many of the movement disorders associated with abnormally low copper (Cumings 1948; Perry 1961; Menkes et al. 1962; Willvonseder et al. 1973; Haas et al. 1981; Parker 1985) and TS (Comings et al. 1984; Devor 1984) have been shown to be compatible with mendelian transmission.

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Visual fields in Gilles de la Tourette Syndrome

SIR: Gilles de la Tourette (GTS) is a complex disorder characterised by multiple motor and one or more vocal tics (DSM-III-R; APA, 1987). It has been shown that GTS is a genetic disorder and the inheritance pattern is consistent with autosomal dominant transmission with incomplete but high penetrance (Eapen, 1993). In such a disorder there is always a quest for biological markers. Enoch *et al* (1988) described anomalous kinetic visual fields in 100% of children with GTS. It was suggested that visual field defects may serve as a genetic marker for GTS. Repka & Singer (1992) performed automated static perimetry on 18 children with GTS. They demonstrated field defects in 25% of cases, however, they observed that these rates approximated to those observed in patients undergoing first time visual field testing.

We undertook a prospective controlled study to which 12 GTS patients (24 eyes), and 12 (24 eyes) age, sex-matched controls were recruited. No ocular disease was detected in any of the subjects, none had previously undergone visual field testing. Visual field tests were performed using a Humphrey Field Analyser running a central 24-2 full threshold test. Data collected included mean deviation (MD) scores, an indication of the overall field abnormality and corrected pattern standard deviation (CPSD) scores, localised field defects. Twenty-one out of 24 visual field tests in each group were reliable according to the reliability indices built into the field analyser software and were included in statistical analysis. There were no statistically significant differences in either MD ($P=0.08$) or CPSD ($P=0.21$) between GTS and control eyes (see Table 1 for results for Humphrey fields). The difference in MD approached significance, and a larger sample might be expected to yield a significant result. However, the MD score for a particular patient would not serve as a biological marker for GTS since there is a large overlap of MD scores in the GTS and control groups. Our study indicates a trend for a higher negative MD score in GTS patients, but we conclude that visual fields do not serve as a useful biological marker.

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Table 1
Results of Humphrey 24-2 fields

	GTS patients					Controls			
	MD		CPSD			MD		CPSD	
Sex/Age	R eye	L eye	R eye	L eye	Sex/Age	R eye	L eye	R eye	L eye
M 22 y	−2.70	−2.02	0.00	0.00	M 23 y	−1.68	−1.64	0.00	0.92
F 46 y	−3.22	−2.33*	2.50	0.00*	F 44 y	0.08	0.16	1.12	0.00
F 51 y	−2.27	−2.36	0.00	4.06	F 50 y	−2.84	−4.63	0.00	5.48
M 23 y	−9.72	−13.9*	7.27	9.60*	M 24 y	−1.46	−1.38	0.00	0.00
M 50 y	−0.70	−1.66	1.38	0.00	M 46 y	−0.24*	0.63	0.52*	0.00
F 52 y	−2.78*	−1.67	1.07*	0.00	F 51 y	−0.77	−1.25	0.00	0.00
M 20 y	−3.40	−1.92	0.00	1.43	M 23 y	−1.29	−1.17	0.39	0.00
F 29 y	−1.69	−2.55	0.00	1.79	F 22 y	−0.78	−1.17	0.60	1.23
M 21 y	−2.10	−0.96	1.26	1.79	M 21 y	−2.33	−0.53	0.00	1.45
M 29 y	−2.58	−2.08	0.97	0.00	M 32 y	−1.26	−0.40*	1.11	1.34*
M 50 y	−2.10	−0.96	1.26	1.79	M 48 y	−1.78	−2.53	1.32	0.00
F 31 y	−3.25	−3.21	0.20	0.00	F 32 y	−8.39	−7.86*	3.75	4.56*

*low reliability score (excluded from statistical analysis).

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ABSTRACTS

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INCREASED PLASMA KYN IN TOURETTE SYNDROME MAY BE DUE TO INDUCTION OF IDO

Gaynor C.M., Rickards, E.H.G., Kariyawasam, S.H., Sidey, F.M., Robertson, M.M., Corbett J.A., Handley, S.L. Dept Pharm. Sci, Aston University, Birmingham B4 7ET, QEPH, Birmingham B15 2QZ and National Hospital, Queen Square, London

Kynurenine (KYN) increases tic-like behaviour in an animal model of Tourette Syndrome (TS) (McCreary & Handley, 1995, J Psychopharmacol 9: 68) and has been reported to be elevated in TS (Dursun et al, 1994, Mol Chem Neuropathol 21: 55). The KYN pathway is the principal route for metabolism of tryptophan (TRY); plasma KYN levels give a gross index of this pathway's activity. In liver, the first enzyme is the cortisol-inducible Tryptophan dioxygenase (TDO), while in extrahepatic tissues, including brain, this is replaced by the cytokine-inducible indoleamine dioxygenase (IDO). Cytokines also induce neopterin formation. Plasma samples were obtained from 72 TS patients and 46 matched controls (C) following an overnight fast. TRY and KYN were measured by HPLC, cortisol by RIA and neopterin was kindly assayed by Prof D Fuchs (Innsbruck). There was no significant change in TRY but KYN levels were significantly elevated (TS: 1.43 ± 0.05; C: 1.29 ± 0.06; μmol/L $p<0.05$). Cortisol was unchanged, while neopterin was significantly elevated (TS: 8.65 ± 0.68; C: 6.89 ± 0.31; nmol/L $p<0.05$). Levels of these substances were not related to current medication. Cortisol was not significantly correlated with KYN in TS subjects or controls, while neopterin was significantly correlated with KYN in controls ($r = 0.454$, $p<0.001$) and even more strongly in TS subjects ($r = 0.613$, $p<0.0001$). These results suggest that the increase in KYN in TS patients is more likely to be due to induction of IDO than TDO, suggesting a possible autoimmune basis for the condition.

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6. Event-related potentials (ERPs) recorded from auditory and visual oddball and auditory selective attention tasks in Tourette's syndrome. – M. Göbel, G. Barrett, Z. Pirtosek and M.M. Robertson (The National Hospital and Middlesex Hospital, London)

Van Woerkom et al. (Electroenceph. clin. Neurophysiol., 1988, 71: 443–447) demonstrated abnormalities of the N1 potential recorded from adult patients with Gilles de la Tourette's syndrome performing a 2-stimulus auditory oddball task. It has been suggested that subjects with Tourette's syndrome have an attention deficit and as the auditory N1 potential is related to attention we sought to extend the oddball result to an ERP test which assesses attention directly.

Thirteen patients aged 15–42 years and 10 control subjects with the same age range performed auditory and visual 3-stimulus oddball tasks and an auditory selective attention task which consisted of a high (1.5 kHz) or low (1.2 kHz) pitch tone delivered to the right or left ear in random order at a rate of 1/sec. All pitch/ear combinations were equiprobable and one was designated as target on separate runs with responses for all 4 possible targets being recorded. Division of attention was evaluated by examining difference wave forms for responses to the same physical stimulus presented to attended and unattended ears.

Results from the oddball tasks suggested that the Tourette's syndrome cases had attentional difficulties and responses in the selective attention task indicated a clear difference from controls suggesting impaired ability to attend to one ear at a time.

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Grip Force Behavior in Gilles de la Tourette Syndrome

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Abstract: We analyzed predictive and reactive grip force behavior in 15 patients with Gilles de la Tourette syndrome (GTS) and 15 sex- and age-matched healthy control subjects. Nine patients were without medication; six patients were on medication. In a first experiment, participants lifted and held instrumented objects of different weight. In a second experiment, participants performed vertical point-to-point and continuous arm movements at different frequencies with a hand-held object. In a third experiment, preparatory and reactive grip force responses to sudden load perturbations were analyzed when a weight was dropped into a hand-held cup either by the subject or unexpectedly by the experimenter. Compared to the healthy subjects, GTS patients had increased grip forces relative to the load force in all tasks. Despite this finding, they adjusted the grip force to changes in load force (due to either a change in the mass lifted or accelerating the mass during continuous movements) in the same way as healthy subjects. The temporal coupling between grip and load force profiles was also similar in patients and healthy controls, and they displayed normal anticipation of impact forces when they dropped a weight into a hand-held cup. We found no significant effect of medication on the performance of GTS patients, regardless of the task performed. These results are consistent with deficient sensory–motor processing in Gilles de la Tourette syndrome. © 2004 Movement Disorder Society

Key words: grip force; object manipulation; predictive force control; Gilles de la Tourette syndrome

Gilles de la Tourette syndrome (GTS) is a developmental neuropsychiatric disorder characterized by multiple motor tics and one or more phonic tics that have to be present for more

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than 1 year.[1–3] Simple tics often relieve internal sensory urges felt in the area of the tic (premonitory sensations) while more complex tics such as echophenomena respond to the perception of external stimuli.[2,3] These clinical observations suggest that GTS is a sensory–motor disorder, for which the sensitivity to external stimuli might be increased and unwanted sensory, motor, or emotional stimuli cannot be sufficiently suppressed. This combination may lead to tics and possibly other features of GTS as habitual motor responses to sensory cues.[2,3]

The pathophysiology of GTS has been associated with the basal ganglia, thalamocortical circuits, and related sensorimotor cortical areas.[4,5] In basal ganglia disorders, such as Parkinson's disease and Huntington's disease, there is often a disruption of serially ordered complex movements.[6,7] In GTS, fast aiming movements were not different to normal controls.[8] However, GTS patients were more reliant on external (visual) cues to execute a motor program during sequential movements,[9] suggesting that they have difficulties in internally cueing their movements and to switch between subsequent motor plans. This explanation would be consistent with the idea that basal ganglia function may be compromised in GTS, because a similar abnormality can be seen in patients with Parkinson's disease.[6,7,10]

Two more recent studies examined the control of grip force during object manipulation in GTS. Flanagan and colleagues examined a single untreated patient and reported normal grip force control during both voluntary movement and during involuntary tics.[11] In contrast, Serrien and coworkers, who examined 9 GTS patients, found they manipulated objects with excessive grip force[12] similar to patients with other basal ganglia disorders, including Parkinson's disease,[13–15] Huntington's disease,[16–18] and dystonia.[19,20]

The aim of the present study, therefore, was to resolve the discrepancy between the work of Flanagan and associates[11] and that of Serrien and colleagues[12] This difference may have been due to the fact that 5 of the patients of Serrien and coworkers were taking medication at the time of the study, whereas the patient of Flanagan and colleagues was untreated. We, therefore, re-examined grip force control in a greater number of untreated GTS patients and compared the results with those in healthy subjects and GTS patients treated with dopamine antagonists.

SUBJECTS AND METHODS

Subjects

A total of 15 patients with a DSM IV diagnosis of GTS (7 women; mean age, 37.1 years; range, 19–62 years) and 15 healthy subjects (7 women; mean age, 37.5 years; range, 19–62 years) participated in the experiments. Nine patients were not on medication for GTS (Table 1). Tic severity was assessed using the Yale Global Tic Severity Scale (YGTSS).[21] All subjects were right-handed, naïve to the specific purpose of the study, and gave informed written consent. All participants performed the experiments with their dominant right hand. The study was conducted in accordance with the Declaration of Helsinki and was approved by the National Hospital for Neurology and Neurosurgery and the Institute of Neurology joint research Ethics committee.

Instrumented Object

Subjects grasped a cylindrical and cordless instrumented object between the fingertips of the dominant right hand. Details about the object have been described elsewhere.[22–24] In brief, the object registered grip force (GF) and linear acceleration in three dimensions. Data were stored within the object and transferred to a personal computer for analysis after each experimental setting with a single subject. For arm movement experiments, the object was used alone (mass, 0.35 kg). For lifting experiments, the object was mounted onto an empty container or a container loaded with a 100-g weight (mass, 0.40 and 0.50 kg, respectively). For the catch-up experiments, the object was mounted onto a cup in which a 100-g weight was dropped (mass, 0.45 kg). Grip surfaces were sandpaper at a medium grain (#240) in all trials performed.

Procedures

Before the experiments, subjects washed their hands with water and soap to remove any excess oils and carefully dried them. Before the experiments began, the experimenter gave verbal instructions, then demonstrated the procedure, and finally observed the subjects as they did the tasks.

TABLE 1. *Clinical details of patients with Gilles de la Tourette syndrome*

Patient	Gender	Age (yr)	Duration of symptoms (yr)	YGTSS	Treatment
V.R.	M	24	14	46/100	None
K.B.	F	19	12	79/100	None
R.C.	M	45	35	26/100	None
D.H.	M	49	44	52/100	None
B.C.	M	30	22	31/100	None
D.B.	F	27	21	59/100	None
K.B.	F	19	13	72/100	None
D.O.	M	32	27	25/100	None
M.L.	M	55	49	40/100	None
A.B.	M	41	35	17/100	Sulpiride (400 mg/day)
J.G.	F	53	42	48/100	Pimozide (4 mg/day)
K.O.	F	19	8	19/100	Sulpiride (400 mg/day)
G.R.	M	41	34	39/100	Sulpiride (200 mg/day)
P.R.	M	41	41	28/100	Sulpiride (100 mg/day)
M.R.	F	62	59	63/100	Haloperidol (1 mg/day)

YGTSS, Yale Global Tic Severity Scale.

Lifting the Object

The subjects sat in a chair in front of a table with their right upper arm parallel to the trunk, and with their unsupported forearm extending anteriorly. The object was grasped between the tips of the thumb and index finger on either side, lifted to 1 cm above the table indicated by a marker, held in this position for 5 seconds, and then replaced and released. Subjects could see the object and their grasping hand and arm during all phases of the task. After 10 lifts with either the 0.40- or 0.50-kg weight, subjects were asked to slowly release the object until it dropped to the support. This procedure was repeated twice to obtain an estimate of the minimal grip force (slip force) necessary to prevent the object from slipping. The slip point was defined as the first detectable change in acceleration along the object's vertical z-axis and the minimum grip force was determined at this time point.

Voluntary Arm Movements

Subjects grasped the object between the tips of the thumb and other fingers. They were instructed to move the object fast between two points 30 cm apart (indicated by a ruler) on a straight, vertical line along its z-axis and to keep the object's orientation constant. During point-to-point movements short breaks of approximately 1 second duration were introduced in between single up and down movements. During continuous (cyclic) movements, there were no breaks and subjects were paced by an auditory stimulus to move the object upward/downward at three different cycle frequencies: 1 Hz, 1.2 Hz, and 1.5 Hz. For both point-to-point and cyclic movements, five trials consisting of 8 to 10 movements with inter-trial breaks of up to 30 seconds duration were performed. At the end of the trials, the minimum grip force required to prevent the object from slipping (slip force) was measured twice for each individual as described above.

Catch-Up Trials

Subjects sat in a stable chair in front of a table with the dominant arm slightly abducted and the forearm held unsupported and rotated in front and in parallel to the trunk with the elbow flexed at approximately 90 degrees. Subjects held the object mounted onto a cup between the tips of the index and thumb. Subjects were instructed to hold the object stationary and to prevent it from slipping. In the experimenter-release condition, subjects were asked to keep their eyes closed for the entire experiment. The experimenter dropped a 100-g weight unexpectedly into the cup from a height of 20 cm indicated by a mark. In a second experiment, subjects themselves dropped the 100-g weight into the cup with their eyes open. Ten such trials with intertrial intervals of 5 seconds were performed for the experimenter- and self-release conditions, respectively. After each experiment, slip forces were determined twice as described above.

Data Analysis

Positive kinematic acceleration (ACC) of the object (along the object's z-axis) was directed upward during lifts and arm movements, but directed downward during catch-up experiments. When the object was held stationary, no acceleration was measured. The net load force (LF) was calculated from the object mass and the vectorial summation of gravity (9.81 m/sec^2) and inertial accelerations along the object's x-, y-, and z-axes. This method included additional inertial loads, which arose from incidental acceleration components in the x- and y-directions.

Lifting the Object

For the last 5 of 10 lifts, (1) maximum kinematic acceleration of the lifting movement (Max. ACC), (2) maximum rate of grip force increase (Max. GF Rate; maximum of the first time derivate of grip force), (3) maximum grip force, and (4) static grip force (Static GF) averaged from a 3-second interval of stationary holding the object were determined. The ratio between maximum grip and load forces was also calculated. Time lags between maximum acceleration and maximum grip force were assessed to quantify the temporal coordination between grip and load force profiles.

For statistical analysis, we first asked whether patients used higher grip forces compared with controls and whether this depended on the load forces. To this end, we used analyses of variance (ANOVA) with the factors "weight" (0.40 kg and 0.50 kg) and "disorder" (GTS patient and healthy control). Next, we examined whether medication had an influence in the GTS patient group. This aspect was tested using ANOVA with the factors "weight" (0.40 kg and 0.50 kg) and "medication" (on medication and off medication).

Point-to-Point Arm Movements

Two time points within the course of voluntary movements were determined: (1) movement start as determined when the acceleration signal deviated more than 2 standard deviations from the baseline level, and (2) maximum kinematic acceleration (Max. ACC; maximum upward acceleration and maximum downward deceleration). At these time points, grip force and acceleration signals, as well as calculated load force were determined. The maximum load force coincided with maximum kinematic acceleration. The grip force at the movement onset provided a measure of grip force established during stationary holding of the object in between single movements. The ratio between grip and load force (Force Ratio) at movement onset and at the time of maximum kinematic acceleration (coinciding with maximum load force) was used to relate the magnitudes of the two forces directly. This force ratio is considered to be a highly sensitive measure of the efficiency of grip force produced in relation to the load force. To describe the stability of the temporospatial coordination between the grip and load force profiles, a correlation analysis between grip and load forces was performed for the entire course of each movement. The average r^2 correlation coefficients were calculated for each subject to assess the regularity and stability of the grip force modulation with the movement-induced load fluctuation. The average slopes and intercepts were calculated for each subject to describe the gain of modulation of the grip force profile with the load force profile.

Statistically, for each movement direction (up or down), we tested whether the grip forces of patients differed from controls using ANOVA with the factors "disorder" (GTS patients or healthy control) and "direction" (up or down). A second ANOVA examined the effect of medication on grip force in the

patient group with the main factors "medication" (on or off medication) and "direction" (up or down).

Continuous (Cyclic) Arm Movements

Data analysis focused on the coupling between the grip and load forces with respect to the level of produced forces and the temporal coordination between the force profiles. To evaluate the scaling of the grip force level in relation to the movement-induced loads, the ratio between grip and load force peaks (Force Ratio) at the time of maximum acceleration (lower turning point of the movement cycle) was determined. To describe the stability of the temporospatial coordination between the grip and load force profiles a correlation analysis between grip and load forces was performed for movement cycles at the three different cycle frequencies.

Statistically, we first asked whether grip forces differed between patients and controls. To test this question, we used ANOVA with the main factors of "disorder" (GTS patients and healthy control) and "frequency" (1.5 Hz, 1.2 Hz, and 1 Hz cycle frequency). In a second ANOVA with the main factors "medication" and "frequency", we examined the influence of medication in the patient group.

Catch-Up Trials

The maximum of load perturbation was only indirectly assessed by determining the maximum of downward acceleration of the cup due to impact. When controlling this measure with an additional load sensor applied between the grip object and the cup, the maximum load indeed occurred synchronously with the maximum peak in downward acceleration. However, the load magnitudes, either measured with the load sensor or calculated from the maximum downward deceleration at impact, did not reliably reveal similar magnitudes. Consequently, we focused our analysis on the temporal measures. We assessed two time points within the grip and acceleration traces of the last five trials in the experimenter- and self-release conditions: (1) maximum acceleration (coinciding with maximum load force) and (2) maximum grip force. The time between maximum grip force and maximum acceleration depends on the predictability of the task and was assessed to provide a measure of the precision of the temporal coupling between grip and load forces.

We used ANOVA to investigate the influence of "disorder" (GTS patients and healthy control) and "condition" (experimenter- and self-release conditions) on the time lags between maximum grip and load force. Next, we tested whether medication had an effect on this parameter using ANOVA with the main factors "medication" (on/off medication) and "condition". A significant difference in the ANOVA was followed by post hoc comparisons using Student's t tests. A P value of ≤ 0.05 was considered statistically significant.

RESULTS

Slip Forces

The average slip forces of GTS patients, irrespective of medication, and healthy controls were similar ($P \geq 0.6$ for all comparisons).

Lifting the Object

In all patients and controls, the maximum force rate and peak force regularly occurred before liftoff. Similar to controls, patients produced a greater peak rate of grip force increase and a greater peak force when lifting the heavier object. In addition, in both groups the static grip force (averaged from 3 seconds of stationary holding) was greater when holding the heavier object. Thus, the patient's ability to scale the grip force output differentially to the different object weights was preserved as previously described in detail by Johansson and Westling[25] in healthy subjects. Medication did not have a significant influence on any of the data in the lifting experiments. Therefore, for all analyses the data from all GTS patients were combined.

There was no difference between patients and controls in the lifting component of the task. The average peak accelerations of the lifting movement were the same in both groups (0.5 ± 0.1 m/sec^2), irrespective of weight ($P \geq 0.8$ for all comparisons). The main difference between the groups was seen in the grip component of the task (Fig. 1). The maximum rate of grip force increase ($F_{1,48} = 7.5$; $P = 0.01$), the maximum grip force ($F_{1,48} = 37.2$; $P < 0.001$), and the static grip force ($F_{1,48} = 27$; $P < 0.001$) were greater in GTS patients compared with healthy controls. We also calculated the ratio of GF to LF. This value was also greater in the patients than the controls ($F_{1,48} = 40.7$; $P < 0.001$, Fig. 1), but it was similar when lifting both weights. This finding suggests that the GF was a constant proportion of the LF in both groups of subjects.

The temporal coupling between the acceleration of the lifting movement and the grip force profile was similarly precise in GTS patients and healthy controls with maximum grip force and maximum acceleration coinciding closely in time. The average time lags between maximum grip force and maximum acceleration were similar for GTS patients (with medication: 10 ± 5 msec and 7 ± 10 msec for lifts with the 0.4- and 0.5-kg weight, respectively; without medication: 16 ± 10 msec and -3 ± 5 msec for lifts with the 0.4- and 0.5-kg weight, respectively) and healthy controls (15 ± 10 msec for lifts with the 0.4-kg weight and 16 ± 12 msec for lifts with the 0.5-kg weight).

Arm Movements

In all our patients and healthy controls, we observed a similar basic relationship between grip force and arm movements as has been reported previously in detail by Flanagan and Wing in healthy subjects.[26] Medication did not have an influence on the data in the patient group. Therefore, for all further analyses, the data of all GTS patients were combined.

Point-to-Point Arm Movements

Patients had higher force ratios at the onset of movement ($F_{1,56} = 10.9$; $P = 0.002$; Fig. 2) and at maximum acceleration ($F_{1,56} = 13.2$; $P = 0.001$; Fig. 2), irrespective of movement direction. A correlation analysis was performed between grip and load force profiles to examine their temporospatial coupling. Despite higher slopes, the correlation coefficient was similar in GTS patients and controls, indicating a very precise and stable temporal coupling between grip and load force profiles in all subjects ($r^2 = 0.78$; SD 0.07 for patients versus $r^2 = 0.78$; SD 0.09 in controls). The gain of grip force mod-

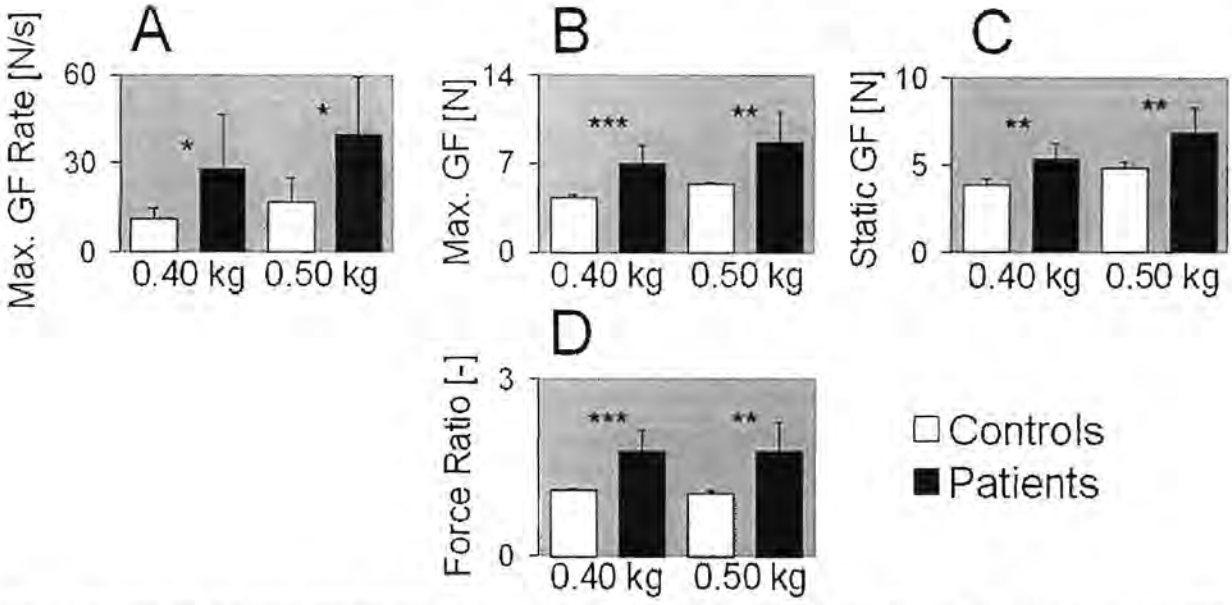

FIG. 1. Grip force rate, grip forces, and force ratios. With either weight, maximum grip force rate (Max. GF Rate, **A**), maximum grip force (Max. GF, **B**), static grip force (Static GF, **C**) and grip force:load force ratios (Force Ratio, **D**) are higher in Gilles de la Tourette syndrome patients compared with controls. Note the different scale in B and C. Data are expressed as means (±SD). The results of post hoc comparisons between patients and healthy controls are indicated (*$P < 0.03$; **$P < 0.01$; ***$P < 0.001$).

ulation with movement associated load fluctuations in both directions was greater in GTS patients than healthy controls ($F_{1,56} = 12$; $P = 0.001$ for slopes and $F_{1,56} = 10$; $P < 0.01$ for intercepts of the least square regression lines). This finding is consistent with the increase in the GF/LF ratios in the lifting experiment. There was no correlation between the amount of grip force overflow and the severity of GTS as measured with the YGTSS.[21]

Cyclic Movements

Grip and load force profiles were tightly coupled for movements at each frequency with their respective force peaks coinciding closely in time. These characteristics have been described in detail by Flanagan and Wing in healthy subjects[27] and were observed in all our GTS patients and healthy controls. There was no difference between treated and untreated GTS patients in any of the different parts of the experiment. Therefore, the data of all GTS patients were combined for further analyses.

As in the other experiments, the grip force component of the task was impaired in patients. Higher force ratios were noted in GTS patients ($F_{1,66} = 124$; $P < 0.001$, Table 2). The force ratios were higher with higher cycle frequencies in both patients and controls ($F_{2,66} = 66$; $P < 0.001$, Table 2). Interest-

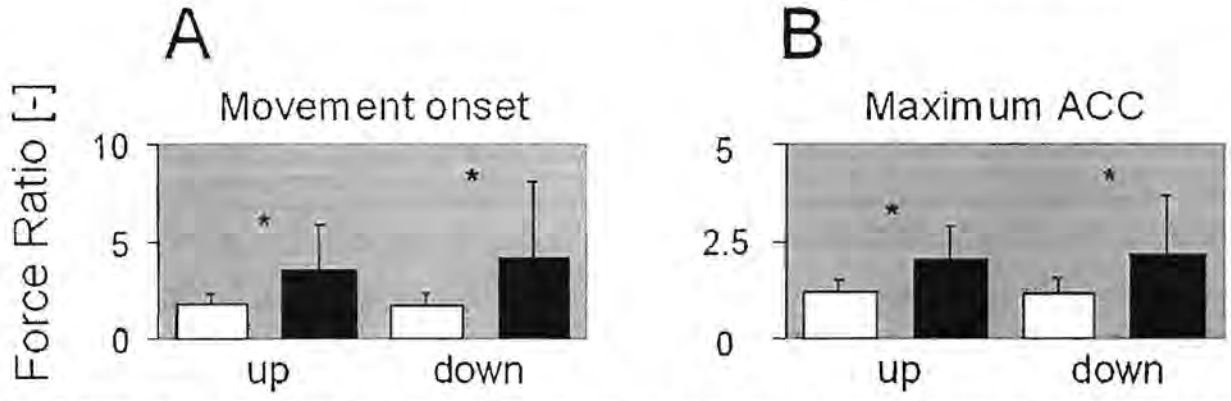

FIG. 2. The ratio between grip and load force at the time of movement onset (**A**) and at maximum acceleration for upward and downward movements (**B**) is greater in GTS patients than controls. Note the different scale in A and B. Data are expressed as means (±SD). The results of post hoc comparisons between patients and healthy controls are indicated (*$P < 0.03$). White bars, controls; black bars, patients.

TABLE 2. *Force ratios (grip force/load force) with cyclical arm movements in patients and controls at different cycle frequencies*

	Cycle frequency		
	1 Hz	1.2 Hz	1.5 Hz
GTS patients	1.4 (0.2)	2.5 (0.3)	3.8 (0.9)
Healthy controls	0.8 (0.1)	1.1 (0.3)	1.7 (0.4)

Values are expressed as mean (SD).
GTS, Gilles de la Tourette syndrome.

ingly, we found an interaction between the factors "disorder" and "frequency" ($F_{2,66} = 14$; $P < 0.001$), indicating that the effect of "frequency" was more pronounced in GTS patients. This finding indicates that, in addition to a higher grip force offset, patients had a greater increase in grip force scaling at higher cycle frequencies. There was no correlation between the amount of grip force overflow and the severity of GTS as measured with the YGTSS.[21]

Catch-Up Trials

The time lags between maximum grip force and maximum acceleration were calculated for GTS patients and healthy controls during both experimental conditions. The average time lags were 106 ± 5 msec and −6 ± 5 msec for patients on medication, 108 ± 15 msec and −8 ± 12 msec for patients off medication, and 105 ± 10 msec and −8 ±19 msec for healthy controls in the experimenter- and self-release condition, respectively. The time lags were larger for the experimenter-release condition than for the self-release condition ($F_{1,56} = 1223$; $P < 0.001$) but not different between patients and controls or between GTS patients on and off medication. Thus, as described in healthy subjects,[28] patients were capable of predicting the load perturbation due to impact and to regulate grip force in anticipation in the self-release condition. In the experimenter-release condition, the perturbation was not predictable and a reactive control mechanism was triggered by impact with the consequence that grip force lagged behind load due to nerve conduction delays.[28]

DISCUSSION

The aim of the present study was to resolve the differences between the results of two previous studies that examined sensory–motor integration in GTS using grip force experiments.[11,12] Consistent with the study of Serrien and colleagues,[12] our results confirm that GTS patients use a higher grip force than healthy controls in all tasks studied. Importantly, we were able to show in a reasonably large group of GTS patients that the increased grip force is very similar between GTS patients on dopamine receptor blocking drugs and untreated patients. This finding indicates that higher grip force in GTS is not an effect of medication. We found no correlation between the degree of grip force offset and the clinical severity of GTS as assessed with the YGTSS. However, because the YGTSS is a global score for rating tic severity, it may not correlate with abnormalities in a particular subset of muscles involved in grip force experiments.

Some tasks involved subjects adjusting their grip to lift and move objects of different weight or accelerate them at different rates. The common feature of the results was that GTS patients were able to adjust grip force to the load force acting on the object being grasped such that the ratio of grip:load force was constant. The same was true in the healthy control group with the only difference being that the ratio was smaller than in the patients. That is, for any load acting on an object, patients exerted a proportionally larger grip force than controls. The results also extended the study of Serrien and coworkers[12] by showing that the timing of predictive and reactive grip force adjustments to sudden expected or unexpected load force changes were also similar to healthy controls. Our results suggest that the motor response (grip force adaptation) to an external sensory cue (load change) has a higher gain in GTS patients than normal and that this difference is responsible for the inefficiently high grip forces that they use. Timing of grip force changes, however, is completely normal. Interestingly, GTS patients increase the ratio of grip:load force more than healthy subjects when moving objects at higher cycle frequencies.

Our data suggest that, in GTS, the sensory information conveyed to the brain about the load acting in an object is translated into an abnormally large motor response, i.e., inefficiently high grip force. However, the detection of changes in sensory input and the execution of the motor programs regulating predictive grip force output were normal. On clinical examination and neurophysiological testing, there is no evidence for sensory abnormalities in GTS.[29–31] Thus, it is more likely that the link between sensory processing in the brain and motor output is abnormal. This explanation would be in keeping with the hypothesis of abnormal gating of sensory information or an impaired inhibitory control of unwanted behavior including motor responses in GTS.[29,32] The increased grip force in GTS is strikingly similar to that reported in other basal ganglia diseases including hypokinetic disorders, such as Parkinson's disease,[13,14] and hyperkinetic disorders, such as Huntington's disease[16–18] and dystonia.[19,20] Abnormal central processing of sensory input has been shown in Parkinson's disease and patients with focal hand dystonia. The gating process of sensory input and the integration with motor programs involves different cortical and subcortical structures as well as their respective input and outflow projections.[29,33] It is conceivable, therefore, that the pathological processes underlying each of the basal ganglia disorders might affect different parts of these circuits, leading to abnormalities common to all.

In conclusion, we confirmed that GTS patients use excessive grip force, whereas the precision of the temporal coupling between grip and load force profiles in both predictive and reactive grip force behavior was similar to controls. This finding provides further evidence to suggest that GTS is a sensory–motor disorder that may result from involvement of basal ganglia-thalamo-cortical circuits. Future research using different tasks linked in with other, e.g., neurophysiological or imaging, techniques might help shed some light on which pathological processes underlie similar deficits of finger force control in different basal ganglia disorders.

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Excitability of motor cortex inhibitory circuits in Tourette syndrome before and after single dose nicotine

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Summary

The pathophysiology underlying the involuntary tics of Gilles de la Tourette syndrome (GTS) remains unknown. Here we used transcranial magnetic stimulation (TMS) to examine the excitability of two different inhibitory systems in the human motor cortex: short interval intracortical inhibition (SICI) and short interval afferent inhibition (SAI) in 10 healthy non-smoking controls and eight untreated non-smoking patients with GTS. Compared with the healthy control group, both SICI (measured at a range of conditioning intensities) and SAI were reduced in patients. This is consistent with the suggestion that reduced excitability of cortical inhibition is one factor that contributes to the difficulty that patients have in suppressing involuntary tics. In addition, the reduced SAI indicates that impaired intracortical inhibition may not be limited to the motor cortex but also involves circuits linking sensory input and motor output. A single dose of nicotine reduced tic severity as assessed by blind video scoring in the majority of patients. In addition, it abolished the difference between patients and controls in SICI and SAI. There was no effect of nicotine, and no difference between controls and patients in measures of motor or SICI threshold. This indicates that cholinergic input can modulate the efficiency of SICI and SAI differently in GTS and healthy controls.

Keywords: transcranial magnetic stimulation; tic treatment; Gilles de la Tourette syndrome; intracortical inhibition; sensory afferent inhibition

Abbreviations: AMT = active motor threshold; CSI = conditioning stimulus intensity; CSP = cortical silent period; FDI = first dorsal interosseus muscle; GCI = global clinical impression; GTS = Gilles de la Tourette syndrome; ICF = intracortical facilitation; ISI = interstimulus interval; MEP = motor evoked potential; MRVS = modified Rush Video Scale; SAI = short interval afferent inhibition; SICI = short interval intracortical inhibition; TMS = transcranial magnetic stimulation

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Introduction

The pathophysiology of Gilles de la Tourette syndrome (GTS), a developmental disorder characterized by several motor and one or more phonic tics present for >1 year, is little understood. Simple tics often relieve internal sensory urges felt in the area of the tic (premonitory sensations) (Bliss, 1980), while more complex tics such as echophenomena respond to the perception of external stimuli. These clinical observations suggest that GTS is a sensorimotor disorder where the sensitivity to external stimuli might be increased, and unwanted sensory, motor or emotional stimuli cannot be sufficiently suppressed (Ziemann *et al.*, 1997; Greenberg *et al.*, 2000). This may lead to tics and perhaps other features of GTS such as habitual motor responses to sensory cues (Leckman, 2002).

There are few electrophysiological studies of GTS. Some have used pre-movement EEG analysis to reveal differences in cortical activity preceding involuntary tics and volitional movement (Obeso *et al.*, 1982; Karp *et al.*, 1996). More recently Ziemann *et al.* (1997) emphasized possible deficiencies in some forms of cortical inhibition. In the motor cortex, they found that patients had reduced short interval intracortical inhibition (SICI) and a short cortical silent period (CSP). The former is thought to reflect excitability in GABAa-ergic

inhibitory systems, whereas the latter may use a GABAergic pathway. The authors suggested that reduced activity in inhibitory systems could be one factor that leads to reduced suppression of involuntarily triggered movements.

The first aim of the present project was to confirm this observation in more detail. Ziemann *et al.* (1997) measured SICI using a paired-pulse transcranial magnetic stimulation (TMS) paradigm with only one conditioning stimulus intensity (CSI). However, we and others recently pointed out that this could produce misleading results since the amount of SICI is dependent on the CSI (Ilic *et al.*, 2002; Butefisch *et al.*, 2003; Orth *et al.*, 2003). If the relationship between intensity and amount of SICI differs in patients and controls, a single measure can give an erroneous estimate of the maximum sensitivity of SICI. Therefore, in this study, we measured SICI at a range of conditioning intensities to test the hypothesis of Ziemann *et al.* (1997) more securely.

The second aim of the project was to extend the measures to another form of cortical inhibition. If some tics are an unsuppressed response to sensory urges, then we might expect to see abnormalities in inhibitory pathways that specifically link sensory input and motor output. One such pathway that can be tested in humans is short interval afferent inhibition (SAI: Tokimura *et al.*, 2000) in which a transient sensory input leads to a rapid and short-lasting inhibition of motor cortex. We added this test to our electrophysiological study of GTS patients.

The third aim of the project was to test whether any of these physiological measures would be of use in a clinical setting. Clinical evaluation of GTS is notoriously difficult because of the variability of tics, and this includes the assessment of any change in tic severity with treatment. Thus, it would be very useful to have a complementary objective measure that could be used to evaluate the effectiveness of new therapeutics. We therefore tested whether differences between patients and healthy controls in measures of cortical inhibition could be normalized by effective clinical treatments. The treatment we chose was nicotine. Several studies have suggested that nicotinic drugs, such as nicotine itself, the psychoactive alkaloid in tobacco, or mecylamine may influence tics particularly in conjunction with haloperidol (McConville *et al.*, 1991, 1992; Silver *et al.*, 2001*a*,*b*). In addition, nicotine is easy to apply and is absorbed rapidly when given as a chewing gum. This allowed for repeated measurements on the same day after a single nicotine dose. Nicotine also has the advantage of being safer to test on healthy control subjects than some of the dopaminergic blocking drugs that are also used to treat Tourette patients such as haloperidol. We evaluated the clinical response using video ratings and investigated whether the TMS measures of motor cortex excitability changed following nicotine.

Material and methods

Patients and control subjects

Nine patients (six men, mean age 31.3 years, range 19–48) with a DSM-IV diagnosis of GTS and 10 control subjects (seven men, mean age 32.6 years, range 24–38) were recruited. In GTS patients, the severity of tics was rated on the day of the experiments using the Yale Global Tic Severity Scale (Leckman *et al.*, 1989); the lifetime history of symptoms indicative of GTS was captured using the diagnostic confidence index (DCI; Robertson *et al.*, 1999) (Table 1). All patients and all control subjects were non-smokers, and no patient was on medication at the time of the study.

Table 1 *Demographic and clinical features of Gilles de la Tourette syndrome patients*

Patient	Gender, age (years)	DCI (%)	YGTSS (%)	Co-morbidity
1	M, 31	41	21	None
2	F, 27	76	66	None
3	F, 33	66	67	None
4	M, 48	47	45	None
5	M, 19	59	52	ADHD
6	M, 30	58	45	ADHD
7	F, 20	52	40	None
8	M, 43	56	33	ADHD, OCD
9	M, 31	62	40	None

DCI = diagnostic confidence index; YGTSS = Yale Global Tic Severity Scale; ADHD = attention deficit hyperactivity disorder; OCD = obsessive–compulsive disorder; M = male; F = female.

Patients gave informed written consent according to the Declaration of Helsinki, and the Joint Ethics Committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery approved the study protocol.

Nicotine assay

Before and ~1.5 h after beginning to chew a nicotine gum (2 mg, Nicotinell, Novartis, West Sussex, UK), 5 ml of venous blood were drawn. Samples were centrifuged at 5000 r.p.m. on a benchtop centrifuge and serum separated into a clean screw top container. Samples were stored at −80°C until they were analysed for nicotine concentration using gas chromatography as described (Feyerabend and Russell, 1990). We had planned to include a placebo condition in which patients were given a non-pharmacologically active gum, but the highly distinctive taste of the nicotine gum meant that patients were readily able to distinguish between the placebo and 'real' gums, so that this approach was abandoned.

Electromyography recordings

Surface EMGs were recorded from the right first dorsal interossoeus (FDI) muscle using silver/silver chloride disc surface electrodes (1 cm diameter) in a belly tendon montage. The EMG signal was amplified and analogue filtered (30 Hz to 3 kHz) with a Digitimer D150 amplifier (Digitimer Ltd, Welwyn Garden City, UK). Data (sampling rate 4 kHz) were digitized for off-line analysis using Signal software (Cambridge Electronic Devices, Cambridge, UK). Peak to peak amplitude of motor evoked potentials (MEPs), the area under the curve of the MEP and the silent period duration were measured with in-house software.

Transcranial magnetic stimulation

Patients and controls were seated in a comfortable chair. They were asked to relax as much as possible. Patients had tics throughout the

experiments but were asked not to suppress their tics. Magnetic stimuli were given with a hand-held figure-of-eight coil (outer winding diameter 9 cm) connected to a High Power Magstim 200 stimulator (Magstim Co., Whitland, Dyfed, UK). This stimulator generates a magnetic pulse with monophasic waveform and in the brain induces a current with posterior–anterior flow when the coil handle is positioned at an angle of 45° pointing backwards. The optimal spot for right FDI stimulation was marked with a felt-tipped pen.

Motor thresholds

Resting motor threshold (RMT) was defined as the minimum intensity needed to evoke an MEP of >50 μV in five out of 10 consecutive trials in the relaxed FDI. Active motor threshold (AMT) was defined as the minimum intensity needed to evoke an MEP of >200 μV in five out of 10 trials in the tonically active FDI (~20% of maximal contraction as assessed visually on an oscilloscope). Thresholds were approached from above threshold in steps of 1% stimulator output. Once no MEPs could be elicited, the intensity was increased in steps of 1% stimulator output until a minimal MEP was observed. This intensity was taken as motor threshold.

Paired pulse paradigm

In each individual, a test stimulus intensity was chosen that elicited an MEP of 0.5–1.5 mV amplitude. The conditioning pulse intensity was varied (60, 70, 80 or 90% of AMT) resulting in four different experimental blocks. With each conditioning pulse intensity and in a randomized order, the 2 and 3 ms interstimulus intervals (ISIs) and the 12 and 15 ms ISIs were examined. The former examine SICI and the latter ICF. With an interval of 4 s between trials, 10 conditioned MEPs were collected for each ISI, and in each experimental block a total of 20 unconditioned MEPs were recorded. The order of data collection for each conditioning pulse intensity was randomized between subjects. Trials recorded while the patients contracted the hand muscles or those coinciding with a tic were excluded on-line. No trials were excluded in the off-line analysis. The average of the amplitudes of each conditioned MEP was expressed as a percentage of the average unconditioned MEP amplitude in the same session. Subjects were asked to refrain from caffeine on the day of the experiment. No major irregularities of sleep, mood or other factors could be elicited by direct questioning.

Cortical silent periods

CSPs were recorded from the tonically active right FDI with the subjects squeezing an object between the thumb and index finger at ~20% of maximum force output. Ten trials at a fixed test stimulus intensity of 150% AMT were collected in each subject with an interval of 4 s between trials. In each individual trial, the duration of the CSP was measured from the beginning of the MEP evoked by the test stimulus to the resumption of (any level of) sustained EMG activity. In addition, the area under the MEP was determined and a ratio of CSP duration/MEP area calculated (Orth and Rothwell, 2004). The gain of the recordings was set to 1 mV/V in order to measure the end of the CSP, and in a second channel was set to 10 mV/V in order to measure the size of the MEP. Gain settings were the same for all experiments.

Short interval afferent inhibition by somatosensory input from the median nerve

SAI of the motor cortex was examined as previously described (Tokimura *et al.*, 2000). In brief, a test MEP of ~1 mV peak-to-peak amplitude was elicited in the FDI by TMS. A paired pulse paradigm examined the influence on MEP size of a supra-threshold electrical stimulus given to the median nerve through bipolar electrodes. The electrical stimulus to the median nerve was delivered at an intensity just above the threshold to elicit a visible contraction in the thenar muscles and preceded the TMS pulse to the FDI hot spot by 14, 18, 20, 22, 24, 26 or 29 ms. Twenty trials of the MEP elicited by TMS alone and 10 trials of conditioned MEPs for each ISI were collected. The amplitude of the MEP in the FDI was measured with in-house software. The average amplitude of the conditioned MEP was expressed as a percentage of the average amplitude of the test MEP alone. Trials recorded while the patients contracted the hand muscles or those coinciding with a tic were excluded on-line. No trials were excluded in the off-line analysis.

Video-recordings

Videos were recorded before and after patients chewed the nicotine gum. Patients were seated comfortably in a quiet room for several minutes Video and audio recordings were then made first of the full frontal body view and then of the head and shoulders only. For each view, patients were filmed for 2 min sitting in the chair with the examiner in the room, for 2 min reading aloud and for 2 min sitting in the chair with no examiner present, so that the total time of the videotaping was 12 min. One of the authors (M.M.R.) who did not know the patients and was blinded to the treatment conditions rated the video recordings.

First, the tic severity on each of the two videos for each patient was compared using global clinical impression (GCI); a difference was rated as one video being 'better' and the other 'worse', or as 'no difference'. Next, for each video, the total number of motor or phonic tics was counted during the 2 min of full body and head and shoulder view with and without the examiner in the room. The total number of motor and phonic tics was related to time and expressed as tics/min. The data were analysed using the Modified Rush Video Scale (MRVS) (Goetz *et al.*, 1999). The MRVS consists of five tic domains: the number of body areas involved with tics; motor tic severity; phonic tic severity; frequency of motor tics; and frequency of phonic tics. Within each of the domains, the severity is rated on a scale from 0 to 4. The sum of the five domain scores provides a total tic impairment score (0–20).

Data analysis

For baseline data examining SICI or ICF, we examined whether there was a main effect of 'intensity' (60, 70, 80 or 90% AMT) on the amount of SICI or ICF. This was tested using analysis of variance (ANOVA) statistically. For SICI, ICF or SAI inhibition, we tested whether there was a main effect of 'ISI' on the size of the conditioned MEP using ANOVA. To test whether controls differed from GTS patients, we also used an ANOVA model examining the main effect of 'condition' on the size of the conditioned MEP. In the same way, we statistically evaluated the CSP data. We repeated this analysis with the data recorded after nicotine.

In both controls and GTS patients, the data before and after nicotine were paired observations. Therefore, in order to examine

whether nicotine had an effect on the size of the conditioned MEP, or on the CSP data, we used a repeated measures ANOVA, with 'time' and 'intensity' as within-subject factors and 'group' as between-subjects factor.

In order to assess the correlation of SICI, or SAI, with clinical data, i.e. tic severity, or nicotine serum concentrations, we used linear regression analysis.

A statistical difference in the ANOVAs was followed by a *post hoc* paired *t* test analysis. Mauchley's test was used to test for sphericity in the repeated measures ANOVAs, and the Greenhouse–Geisser correction applied to the degrees of freedom if necessary. Statistical significance levels were set to $P = 0.05$. All statistical analysis was performed using SPSS 11 for Windows software package.

Results

Motor thresholds

Control subjects and GTS patients had similar mean AMTs and RMTs (Table 2). Nicotine had no effect on thresholds (Table 2).

Intracortical inhibition and facilitation

The theoretical threshold for SICI and ICF was extrapolated as described (see Material and methods; Orth *et al.*, 2003). This was not possible in one control subject for SICI and in two control subjects for ICF because the data were too variable. The threshold of both SICI and ICF expressed as a percentage of each individual's AMT was similar in controls and GTS patients, and was unaffected by nicotine (Fig. 1A and B).

We went on to examine how SICI or ICF varied at different CSIs (60, 70, 80, 90% AMT). An increase in the CSI increased the effectiveness of both SICI and ICF (Fig. 2A–D). We analysed the data from SICI or ICF separately in two three-way repeated measures ANOVAs, with 'time' and 'intensity' as within-subject factors and 'group' as between-subject factor. For SICI, there was, as expected, a main effect of 'intensity' [$F(3,51) = 15.9$, $P < 0.001$] but also a significant interaction between 'time' and 'group' [$F(1,17) = 5.871$, $P = 0.027$]. This was due to the significantly greater amount of SICI in controls than in GTS patients before nicotine [two-way ANOVA, main effect of 'group' on the data of Fig. 2A; $F(1,68) = 6.9$, $P = 0.011$]. After nicotine, both groups behaved similarly (two-way ANOVA, main effect of 'group' on the data of Fig. 2B; not significant). We then examined the effect of nicotine in controls and GTS patients separately. This revealed that nicotine did not have a significant effect when either group was tested alone (two-way repeated measures ANOVA).

We performed a similar analysis for ICF. There was a significant increase of the amount of ICF with increasing CSIs [repeated measures ANOVA, main effect of ISI, $F(3,51) = 3.8$, $P = 0.015$]. There was no significant effect of nicotine (no main effect of 'time', $P > 0.05$, no interaction of 'group' and 'time').

Table 2 *Motor thresholds*

	RMT		AMT	
Nicotine	–	+	–	+
Controls	38.7 (6.2)	38.7 (5.7)	29.3 (6.8)	29.3 (6.8)
GTS	41.8 (8.1)	41.7 (8.2)	30.9 (6.9)	30.6 (6.8)

There was no significant difference between the motor thresholds of patients with Gilles de la Tourette syndrome (GTS) and controls. Data are means (SD) from nine GTS patients and 10 controls. RMT = resting motor threshold; AMT = active motor threshold.

Short interval afferent inhibition

In controls and GTS patients, a supra-threshold electrical stimulus to the median nerve at the wrist before the TMS pulse to the FDI hot-spot reduced the mean amplitude of the test stimulus predominantly at ISIs of 20, 22 and 24 ms (Fig. 3A). Figure 3B shows the time course of SAI after nicotine. It appears that the initial difference between GTS

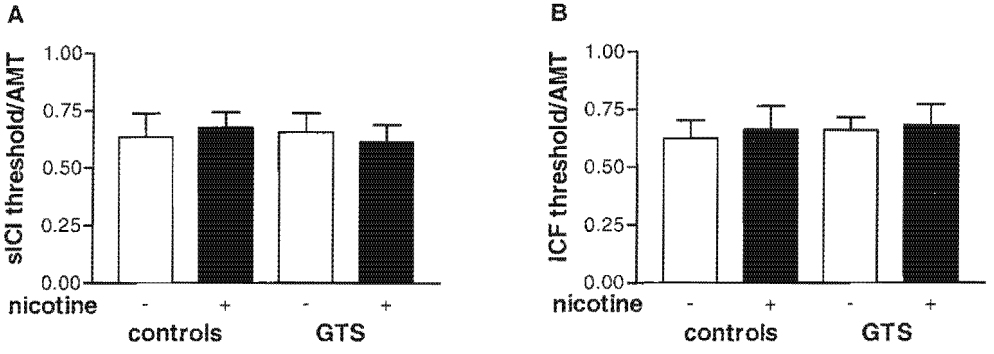

Fig. 1 Thresholds for SICI and ICF. There was no difference between the ratios of the theoretical thresholds for SICI or ICF, respectively, and AMT between patients with GTS and control subjects. In both groups, these ratios remained similar in the presence of nicotine (black bars) compared with baseline values (open bars). Values are means ± SD, $n = 9$ for controls and GTS patients for SICI; $n = 8$ for controls and $n = 9$ for GTS patients for ICF.

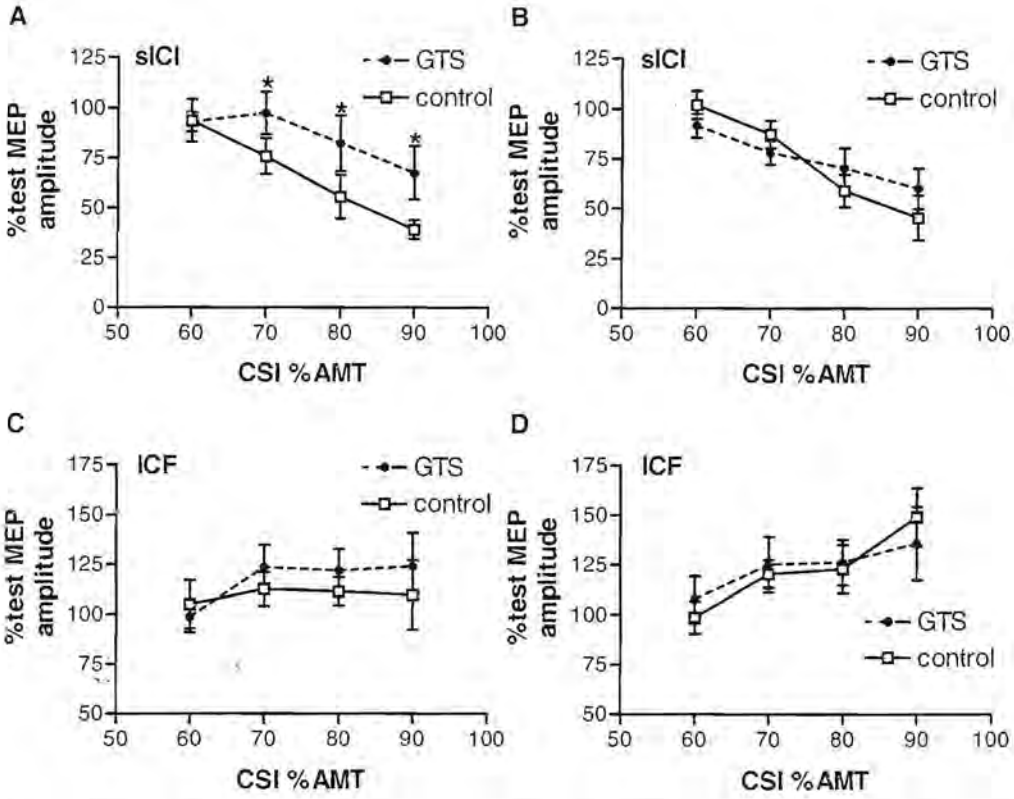

Fig. 2 SICI and ICF with different CSIs. (**A**) Control subjects and GTS patients had increasing amounts of SICI with increasing CSI (main effect of intensity, repeated measures ANOVA, $P < 0.001$) but patients had less SICI (main effect of group, ANOVA, $^*P = 0.011$). (**B**) Nicotine markedly reduced the difference between GTS patients and controls. (**C**) With increasing CSI, the amount of ICF increased (main effect of intensity, repeated measures ANOVA, $P = 0.015$); however, there was no statistical difference between GTS patients and controls. (**D**) With nicotine, controls had more ICF than without but this difference was not significant. Values are means ± SEM, $n = 9$ for GTS patients, $n = 10$ for controls.

patients and controls in the amount of inhibition was abolished after nicotine. Since the early period of inhibition is more likely to have a partly cortical origin than later timings (Tokimura *et al.*, 2000), we assessed the maximum amount of afferent inhibition in each individual. At baseline, GTS patients had significantly less inhibition than controls [ANOVA, $F(1,17) = 9.7$, $P = 0.006$, Fig. 3C]. Nicotine had a different effect on GTS patients and controls; a two-way repeated-measures ANOVA with 'time' as within-subject factor and 'group' as between subject factor revealed a significant interaction between 'group' and 'time' [$F(1,17) = 8.0$, $P = 0.012$, Fig. 3C]. Following nicotine, the difference between controls and GTS patients disappeared (no main effect of group, $P > 0.1$). We then analysed the effect of nicotine separately in controls and GTS patients. This revealed that there was no significant effect of nicotine on maximal SAI in controls or GTS patients (Fig. 3C).

Cortical silent periods

We analysed the duration of the CSP and the MEP area, and calculated the ratio (duration)/(MEP area) as described previously (Orth and Rothwell, 2004). We analysed data using a two-way repeated measures ANOVA, with 'time' as within-subject factor and 'group' as between-subject factor. This revealed that nicotine increased the MEP area [$F(1,17) = 5.081$, $P = 0.038$, Fig. 4A] with a similar effect in both groups (no significant interaction between 'group' and 'time). There was a tendency for MEP area to be smaller in GTS patients, but this was not significant (no main effect of 'group', Fig. 4A). There was no significant difference between the groups and no significant effect of nicotine on either CSP duration or the ratio (duration)/(MEP area) (Fig. 4B and C), although CSP duration was slightly shorter in patients as reported by Ziemann *et al.* (1997).

Behavioural effect of nicotine

No participant had side effects from the nicotine chewing gum or the TMS. No blood sample taken before the nicotine chewing gum contained nicotine. After the chewing gum, controls had mean nicotine plasma concentrations of 4.4 ng/ml (SD 1.26). This was similar to GTS patients (mean 4.2, SD 1.62). The analysis of the videos revealed that on GCI, tics were better following nicotine in six patients, while

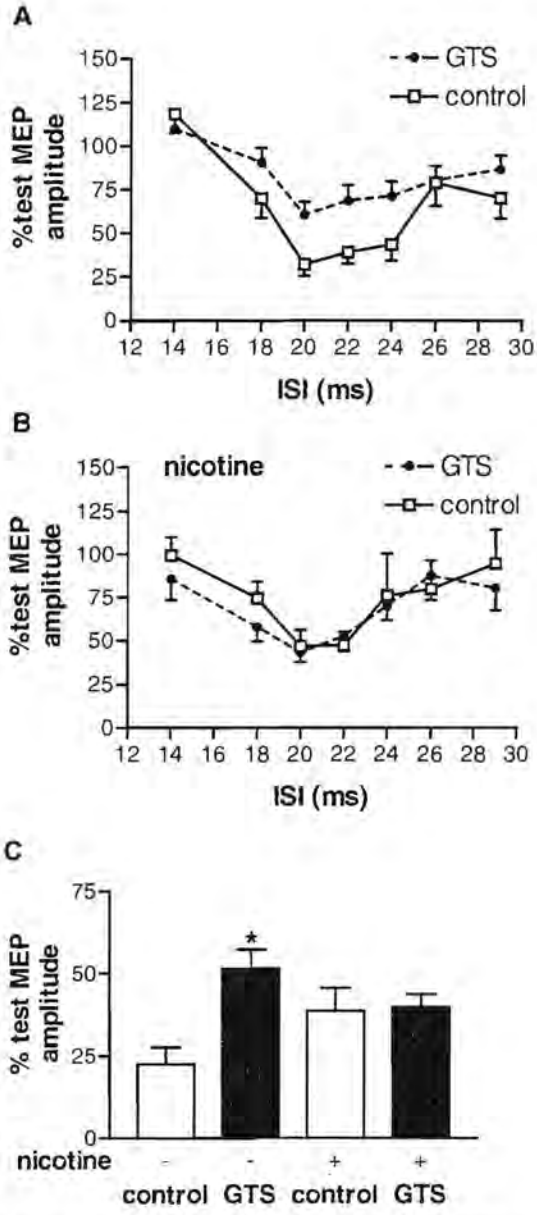

Fig. 3 Short interval afferent inhibition curves. (**A**) In the absence of nicotine, both controls and GTS patients showed significant inhibition at ISIs of 20, 22 and 24 ms (repeated measures ANOVA, $P = 0.001$). (**B**) In the presence of nicotine, the amount of inhibition was similar in controls and GTS patients. (**C**) The analysis of the cortical inhibitory effects at maximum inhibition reveals that patients had less inhibition than controls ($^*P = 0.006$). Nicotine had a different effect in controls and GTS patients (repeated measures ANOVA, interaction between 'group' and 'time', $P = 0.012$). In the presence of nicotine, controls had less inhibition while GTS patients had more inhibition, but these effects were not significant. Values are means ± SEM, $n = 9$ for GTS patients, $n = 10$ for controls.

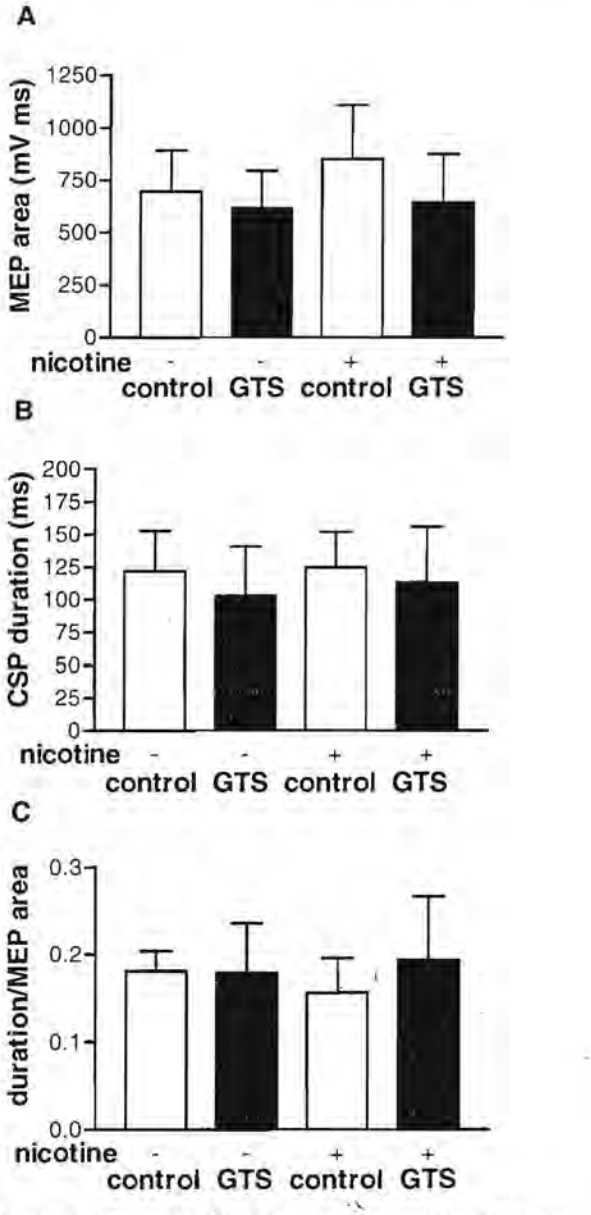

Fig. 4 Cortical silent period. (**A**) Nicotine increased the MEP size (repeated measures ANOVA, $P = 0.038$) but the effect was similar in controls and GTS patients. (**B** and **C**) Silent period duration (**B**) and the ratio (MEP size/CSP duration) (**C**) were similar between controls and GTS patients, and there was no significant effect of nicotine. Values are means ± SD, $n = 9$ for GTS patients, $n = 10$ for controls.

in two patients there was no change and one patient was worse. MRVS total scores revealed a mild but significant improvement of the objective tic video analysis [mean 11.2 (SD 1.7) before versus 10 (1.4) after nicotine, paired samples t test, $t = 3.1$, $P = 0.016$].

Correlation of clinical parameters with TMS parameters and nicotine readings

We expressed the nicotine-induced change of electrophysiological parameters, i.e. SICI and SAI, or tic severity as judged by video scores, as a percentage of baseline (pre-nicotine) measures (see Table 3). The percentage change of SICI or SAI did not correlate with the percentage change of tic severity (linear regression analysis). We then correlated the percentage change of either SICI, SAI or tic severity with serum nicotine concentration. This revealed that there was no

Table 3 *Effect of nicotine on electrophysiological parameters and tic severity in individual GTS patients*

Patient	SICI	SICI nicotine	SAI	SAI nicotine	Tics GCI	MRVS (%change)	Nicotine (ng/ml)
1	61.4	67.6	79	41.8	Better	9.1	5.2
2	58.9	55.3	47	49.9	Better	9.1	7.2
3	28.4	66.4	51.4	26.3	Better	20	2.4
4	32.5	22.1	61.1	50.1	Same	8.3	2.3
5	110.4	67.6	70.4	48.2	Better	16.7	5.4
6	116.6	53.4	56.5	21.2	Same	0	2.9
7	21.8	14.4	37.1	52.9	Better	0	4.5
8	49.4	43.9	34.6	30.9	Better	30	4.4
9	70	45.9	25.5	35.2	Worse	0	3.3
Pooled controls	32.5 (15)	35 (18.6)	26 (18.1)	38.6 (21.8)			

SICI and SAI are expressed as a percentage of unconditioned MEP. Values represent the maximum SICI obtained with different conditioning stimulus intensities, or the maximum SAI with different interstimulus intervals as described in Material and methods. The data of controls were pooled for comparison; values are the means (SD). SICI = short interval intracortical inhibition; SAI = short latency afferent inhibition; GCI = global clinical impression= MRVS: modified Rush Video Score.

correlation of any of these parameters. Next, we looked at those patients that improved by GCI on tic rating. Improvement of any of the electrophysiological parameters did not correlate with clinical change (Table 3).

We went on to evaluate the responses of individual patients. To this end, we took the maximum SICI or SAI of controls and calculated the mean and the SD. We then rated the patients' maximum measures of SICI or SAI as either 'normal' or 'abnormal' (greater than the mean plus 1 SD; see Table 3). At baseline, six patients had 'abnormal' SICI and six patients had 'abnormal' SAI; only one patient had both 'normal' SICI and SAI (see Table 3). We then repeated this with the data after nicotine. First, we looked at how many patients were now in the 'normal' pre-nicotine range; four patients were now 'normal' for SICI and five for SAI. Only one patient still had both 'abnormal' SICI and SAI, but only two patients had both 'normal' SICI and SAI (Table 3). We then calculated the mean and SD of controls after nicotine and rated the patient data. This showed that five patients were now in the 'normal' range for SICI, and all patients were now in the 'normal range' for SAI.

Discussion

The present study shows that the excitability of SICI is reduced in GTS patients at all intensities of conditioning stimulus. In addition, we show that a measure of inhibitory interactions between afferent input and motor ouput, SAI, is also reduced. A single dose of nicotine, at serum nicotine levels similar to those seen after smoking a single cigarette, adjusts electrophysiological measures of the excitability of circuits within the motor cortex to normal levels in GTS and reduces tics.

Baseline (pre-nicotine) electrophysiology in GTS patients

As reported previously (Ziemann *et al.*, 1997), we found that in the basal state (pre-nicotine), motor thresholds of untreated and non-smoking GTS patients were similar to those of controls and that SICI was smaller than normal. In addition, the duration of the CSP tended to be shorter in patients than in controls, but this was not significant in our group of individuals. The shorter CSP duration was accompanied by a smaller MEP size in patients so that the ratio of CSP duration/MEP size was similar in patients and controls (Orth and Rothwell, 2004).

Our results extend previous data in two ways. First we distinguished between the threshold intensity needed to produce SICI and the amount of SICI at suprathreshold intensities of conditioning shock (Orth *et al.*, 2003). This showed that patients had normal thresholds for SICI, but that recruitment of inhibition at suprathreshold intensities was reduced. It is thought that TMS pulses recruit SICI by exciting axons and that this secondarily leads to synaptic release of inhibitory neurotransmitters. Thus, normal thresholds in the presence of decreased recruitment would be compatible with the idea that in GTS, axonal excitability is normal whereas the recruitment of synaptic inhibition in the SICI circuit is reduced. Effectively, the motor system might use SICI to shape patterns of motor output. If the sensitivity of this system were reduced, then a given input would lead to less effective output, and hence less effective shaping of the motor command.

The second new result was that our electrophysiological measure of inhibitory interactions between sensory input and motor output, SAI, was reduced in the baseline state in patients. Again this is consistent with a reduced efficiency of synaptic inhibition. Given the possible influence of sensory inputs in triggering the release of tics, our electrophysiological data suggesting impaired sensory motor inhibition may be a direct physiological reflection of increased access of sensory input to motor output in GTS. In essence, we imagine that sensory input can influence motor output through a variety of channels, some inhibitory and some excitatory. If the motor system needs to reduce the possibility of sensory input triggering movement, then it may be necessary to shift the balance of excitability towards inhibition. If some of the

inhibitory pathways (such as SAI tested here) are less responsive than normal, a given input will lead to less effective suppression of sensory influences than expected, and may contribute to release of involuntary movements.

However, it is important to note that a deficit in these two inhibitory pathways at rest does not preclude the possibility that input from other areas of the brain can compensate for their function, at least temporarily. It is very clear, for example, that patients can sometimes perform at extremely high levels of efficiency despite this lack of inhibition. Patients can also suppress their tics with effort of will and, in these conditions, imaging studies show activation of circuits linking striatum, frontal lobe and those cortical areas involved in movement execution (Peterson *et al.*, 1998; Stern *et al.*, 2000). EEG-coherence analysis indicates that movement inhibition increases cortico-cortical coupling more in GTS patients compared with normal controls, suggesting that the increased activity may compensate for abnormal input into the motor cortex (Serrien *et al.*, 2005). The degree to which patients are able to suppress their tics may thus reflect the balance between underlying deficits and adaptive, compensatory changes in other parts of cortico-subcortical networks involving the basal ganglia, motor and pre-motor cortex, thalamus and pre-frontal cortex. Much as tics take a waxing and waning course in intensity, and can occur in bouts, the interactions between different parts of these cortico-subcortical networks, and ultimately their influence on shaping motor output, are not static but change continuously. Curiously, many patients experience that engaging in specific tasks can abolish their tics, and the preceding urges, altogether and thus enable them to perform normally in highly complex and demanding motor activities; it seems therefore that activity in brain areas relevant to attention such as the prefrontal cortex may have a particularly strong compensatory influence on motor output via complex neuronal networks.

Effects of single nicotine dose

The present data confirm that a single nicotine dose in GTS can reduce tic severity in the majority of the patients tested. However, while significant, the improvement was small and it is not certain that this will be clinically meaningful. In addition, the highly distinctive taste of the nicotine chewing gum meant that we were not able to include a blinded control condition so that a placebo effect could have contributed to the clinical effect that we observed. Therefore, while our data are in agreement with previous studies showing that modulation of the acetylcholine system may be promising as a treatment for GTS (Silver *et al.*, 2001*a*,*b*), we do not think that our data provided conclusive evidence to recommend nicotine chewing gum. This study, assessing the short-term effect of a single low dose of nicotine, was not intended to evaluate nicotine as a treatment for tics; this needs to be the subject of further research. Thus, although it is interesting to note that overall the electrophysiological data were consistent with the clinical results, we also found that there was no correlation in individual patients between improvements in clinical and electrophysiological scores. This suggests either that there is too much variation in the scoring systems to obtain significance with the numbers of patients that we examined, or that other factors, such as a placebo effect, may have influenced the clinical response. Further studies are needed to address these questions perhaps using other agents with an established effect on tics such as dopamine receptor antagonists.

While it is conceivable that the clinical effect of nicotine may be due to a placebo effect, we think it is unlikely that such a placebo effect could account for the effect on SICI or SAI that we measured because clinical and electrophysiological effects did not correlate. However, placebo may increase release of striatal dopamine (de la Fuente-Fernandez *et al.*, 2001), suggesting that placebo may in some circumstances even be able to modify physiological measures. Future studies will be required to address this point.

The electrophysiological data suggest (i) that these two inhibitory pathways in the motor cortex can be modulated by cholinergic inputs; and (ii) that this effect differs between GTS patients and normal controls. There is good evidence from *in vitro* studies in tissue of animals and humans for cholinergic modulation of inhibitory pathways in the brain. Nicotinic and muscarinic acetylcholine receptors (AchRs) are widely distributed throughout the human CNS (Kimes *et al.*, 2003; Podruchny *et al.*, 2003) and the effects mediated at these receptors not only have direct synaptic effects themselves but also modulate synaptic transmission at GABAergic and glutamatergic synapses (McCormick and Prince, 1985; Lena *et al.*, 1993; Jones and Yakel, 1997; Lena and Changeux, 1997; Fisher *et al.*, 1998; Xiang *et al.*, 1998; Zhong *et al.*, 2003). Indeed, recent *in vivo* experiments in humans have shown that the muscarinic antagonist scopolamine reduced SAI, consistent with cholinergic modulation of inhibitory transmission (Di Lazzaro *et al.*, 2000). Although Di Lazzaro *et al.* (2000) did not find any effect of scopolamine on SICI, they did not test for nicotinic effects on either SICI or SAI.

If the effects on SICI and SAI in the present experiments are due to a cholinergic action of nicotine on the excitability of two classes of inhibitory interactions in the motor cortex, then the fact that differences between patients and controls can be improved by administration of nicotine suggests that there is no structural deficiency in these connections in patients. This would be consistent with the normal threshold for recruitment of inhibition. This implies that the deficiencies in GTS are likely to be caused by a subtle loss of neuromodulatory function in cortical circuits.

Our finding that cholinergic stimulation can remove the neurophysiological differences between GTS and controls does not necessarily indicate that GTS is primarily due to cholinergic deficits. Others, for example, have suggested a role for dopamine. However, since both transmitters can serve as neuromodulators, it is conceivable that one can substitute

to some extent for the other or that the functional cholinergic deficit in GTS patients is a consequence of abnormal neuromodulation by dopamine. Clearly, further studies are needed to address this issue more fully.

In conclusion, we show that in GTS patients, inhibitory control over motor output was reduced. This was not restricted to SICI, a measure of motor–motor inhibition, but included SAI, thus supporting clinical observations indicating a role for sensory symptoms in provoking tics. Nicotinic modulation of inhibitory cortical pathways differed between controls and GTS patients and provides further insight into possible mechanisms that underlie these physiological changes.

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Attentional Deficits in Gilles de la Tourette Syndrome

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Summary: Both clinicians and patients with Gilles de la Tourette syndrome (GTS) describe difficulties in everyday life, related to attentional problems. Yet little investigation of these has been carried out using behavioural measures. The present study compares adults with GTS and control subjects on a range of clinical and experimental measures of attention; self-report measures of mood, anxiety, and obsessionality were also employed. Although the GTS group was significantly more depressed, anxious, and obsessional, performance on the attentional measures tended not to correlate significantly with these. Deficits in attention were found in the GTS group on several of the more complex tasks, including serial addition, block sequence span (forwards), the trail-making test, and a letter cancellation vigilance task. Possible explanations and implications for future work are considered. Key Words: Tics—Attentional processes—Experimental tasks—Behavioural measures. NNBN 5:170–177, 1992

Gilles de la Tourette syndrome (GTS) is a movement disorder characterised by involuntary motor and vocal tics. Simple tics occur most frequently in the facial muscles, and patients also demonstrate a variety of complex movements and vocalisations. While the aetiology of the disorder has not been clearly established, there is now widespread belief that it has an organic basis. Some research has reported a high proportion of central nervous system abnormalities in patients (1), but others have not supported this (2). Neurotransmitter abnormalities, probably in the dopaminergic system, have been implicated (3).

Studies have usually reported the distribution of intelligence of GTS subjects to be within the normal range (4, 5). A review by Golden (6) examined the findings of a number of studies of cognitive function in GTS subjects. Whereas language skills are commonly reported to be largely unimpaired, visuospatial deficits have been documented by a number of authors. However, the extent to which difficulties in focusing or sustaining attention might play a role in contributing to deficits on a variety of tasks has not been investigated in detail. Golden (6) pointed out that, despite an apparently high prevalence of attentional deficits in GTS, few studies have employed appropriate control groups to investigate this.

Problems in school are frequently reported, with sufferers falling behind their peers despite showing normal intellectual ability. Robertson (7) drew attention to the fact that associated features of GTS such as learning disabilities, hyperactivity, and attention deficit disorder (ADD) may often be the problems that first bring the patient to medical attention. Erenberg et al. (8) reported learning problems in 36% of a sample of 200 children with GTS. Comings and Comings (9) assessed attentional deficits in GTS by questionnaire, and concluded on the basis of patients' or parents' impressions that 62% of the GTS children had attention deficit disorder compared with 6.3% of controls; higher rates of special teaching requirements were also found among the GTS children. A similar or possibly higher rate of ADD has been reported by others (10, 11). Comings and Comings (12) noted that some school problems may arise because the tics and vocal noises themselves are so distracting; in some cases, the tics were so severe that it was difficult for the children to keep their eyes on what they were reading. They also described subjects' reported difficulties

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with filtering out extraneous noise and increased agitation on timed tasks. Hagin et al. (13) studied 10 children with GTS using a neuropsychological battery. They reported that all but one of the children were able to carry out the more complex tasks of the trail-*making* test, which requires sequencing and sustained attention, but, since a control group was not employed, it is unclear whether performance was in the normal range. They also reported on an auditory discrimination test, where the majority of children performed below average; they stated that poor performance might have been due to either poor auditory discrimination or difficulties in sustaining attention. The authors concluded overall that children with GTS showed fairly generalised problems in focusing attention on salient stimuli and in sustaining responses over time.

More information is needed about attentional processes in GTS in order to further our understanding of the nature of the disorder, and the difficulties such patients experience in everyday life. In the absence of a clear-cut theoretical model of neuropsychological function in GTS, the present descriptive study was designed as a preliminary investigation to explore attentional processes in greater detail, using behavioural measures of attention.

METHOD

Subjects

Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition Revised (DSM-III-R) criteria (14) were used to select 19 right-handed subjects with GTS (12 men, 7 women), aged between 18 and 65, with English as their first language. They were recruited from both outpatient facilities at a specialist GTS clinic, and the United Kingdom Tourette Syndrome Association, and indeed many belonged to both; our clinical experience indicated that the severity of symptoms in the cohort studied was not markedly different from the two referral sources. The GTS subjects were of mean age 32.74 (SD 13.15) and mean full-scale IQ 107.42 (SD 21.10). Eight of them were taking dopamine antagonists to control their motor and vocal tics (haloperidol, sulpiride, or pimozide); four were taking serotonin reuptake inhibitors for depressive or obsessional symptoms (fluvoxamine, clomipramine, or fluoxytene). General exclusion criteria were any significant history of alcohol or drug abuse, physical illness or other psychiatric disorder. One subject was excluded, since his motor tics were so severe that they disrupted his ability to make written responses on the tests. Similar exclusion criteria were used to select the 22 control subjects (7 men, 15 women), of mean age 29.73 (SD 10.71) and mean full-scale IQ 108.00 (SD 14.16). The t tests showed that the groups did not differ significantly in age or IQ, nor did sex ratios differ significantly using a chi-squared test.

Clinical Measures

The Wechsler Adult Intelligence Scale–Revised (WAIS-R), or a shortened form (15) was used to provide an estimate of full-scale IQ; in a few cases where subjects had recently been tested on the WAIS, a subtraction of 7 IQ points was made from full-scale IQ for comparison with the WAIS-R. The following standardised self-report measures were also administered to assess depressed mood, anxiety, and obsessionality: the Beck Depression Inventory (16), the State-Trait Anxiety Inventory (17), and the Leyton Obsessional Inventory (18).

Experimental Measures

Paced and Unpaced Auditory Serial Addition Test

The PASAT tests ability to sustain concentration while performing mental arithmetic on items held in working memory. The original task presented a series of 31 audiotaped digits at four different speeds, and required subjects to add pairs of digits so that each new digit is added to the immediately preceding one (19). Similar series of digits were used in the present task, with slightly slower speeds of presentation, following Gotham et al. (20). After practice, subjects first performed an unpaced task, where they were given the next digit as soon as they had given the answer to the sum of the previous two digits. Two audiotaped paced series were then presented, with intertrial intervals of 4 and 2 seconds, respectively. Correct and incorrect responses were recorded.

Block Sequence Span

The block sequence span is a working memory task that measures span of immediate recall for a spatial sequence [Corsi, reported by Milner (21)]. It consists of nine black 1.5-in. cubes attached in a fixed random pattern to a black base. The examiner tapped the blocks in a prearranged sequence at the rate of 1 per second, and the subject was then asked to copy the pattern. The trials were increased in length every second trial, until the subject failed two trials of the same

length. A second series was then performed where the subject was required to tap the blocks in the reverse order to that of the examiner. The number of correct forward and backward sequences was recorded.

Stroop Test

The Stroop task presents colour words written in conflicting colour inks (22), and measures the efficiency with which subjects can inhibit responding to one of the two conflicting attributes in the same stimulus. Subjects were presented with groups of letters 0.5 cm high, stencilled in coloured ink (red, blue, green, or black) in columns onto an A4 size card; no colour appeared twice consecutively in each column. A control colour Stroop card comprised the letter O written in a series of four in place of words. A conflicting colour Stroop contained the words "red", "blue", "green", or "black". written in ink of a conflicting colour. In each condition, the subject was asked to go down the columns naming the colours of the inks, as quickly as possible, but without making errors. Both overall colour-naming time and errors were recorded.

Letter Cancellation Test

In vigilance tasks of the letter cancellation test type, subjects are required to sustain attention in cancelling out target stimuli from among an array of distractors (23). The present task consisted of six rows of visually presented letters, with no single letter appearing more than once consecutively. Target letters were randomly interspersed with distractor letters, and identical stimuli were used for two conditions. In the first, subjects were asked to work along the rows, crossing out a single target letter ("H") as quickly as possible, without missing any. In the second condition, there were two targets to cross out ("C"s and "E"s). Overall time taken and errors were recorded.

Trail-Making Test

The trail-making test, described by Reitan (24), requires subjects to sustain attention while locating series of targets and alternating between them. The first part of the test consists of 25 numbered circles, and the subject is required to draw a line connecting them in ascending order. In the second part, the circles contain both letters and numbers, and the subject is asked to draw a line alternating between numbers and letters, again proceeding in ascending order. Appropriate examples are given, and the subject is asked to work as quickly as possible, without errors. Any errors made are pointed out to the subject for correction, and the total time to complete each part of the test is recorded.

Procedure

After informed consent had been obtained, participants carried out the experimental and clinical tasks. Order of presentation of tasks was counterbalanced within each group to control for any effects of anxiety or fatigue. The self-report questionnaires were administered after the testing session.

Design

A between-subjects repeated measures design was used to compare two groups, those with GTS and normal controls. The groups were compared using repeated measures analysis of variance, and post hoc tests as appropriate.

RESULTS

The mean scores and standard deviations for the GTS and control groups for each measure are shown in Table 1. A significance level of 5% was adopted throughout, with appropriate alpha adjustment (alpha divided by number of tests) when post hoc tests were performed.

Paced and Unpaced Auditory Serial Addition Test

The PASAT data were transformed logarithmically to reduce skewness. Repeated measures analysis of variance was performed to compare the groups on the three tasks, unpaced, 4-second and 2-second serial addition, with one between-subjects factor (group) and one within-subjects factor (task). The group × task interaction was not significant; there was a significant effect of group ($F = 5.05$, $df = 1, 39$, $p < 0.03$). Post-hoc t tests showed a near-significant group difference in each task, unpaced ($t = 2.04$, $df 39$, $p < 0.05$), 4-second ($t = 1.74$, $df = 39$, $p < 0.09$) and 2-second serial addition ($t = 1.83$, $df = 39$, $p < 0.08$). An inspection of the mean scores showed that the GTS group tended to make more errors than the controls in each condition. Within each group, the mean scores showed little difference in error rates between the unpaced and 4-second tasks, but a faster speed of presentation (2 seconds) increased the error rates markedly for both groups. To examine any effects of sex, age, or IQ, Pearson correlation coefficients were calculated within each group for each of the variables with the

TABLE 1. *Means for the GTS and control groups on the experimental and self-report measures*

	GTS		Controls	
	Mean	SD	Mean	SD
Experimental measures				
PASAT errors				
Unpaced	6.16	(7.81)	2.59	(3.92)
4 seconds	6.63	(7.69)	3.05	(2.32)
2 seconds	14.42	(6.35)	10.50	(3.71)
Block span				
Forward	5.26	(0.99)	6.14	(1.04)
Backward	5.21	(1.13)	5.45	(0.86)
Stroop control (seconds)	54.79	(12.62)	52.27	(6.76)
Errors	0.16	(0.50)	0.23	(0.61)
Conflicting colour (seconds)	101.26	(40.47)	85.95	(14.98)
Errors	1.84	(5.03)	0.27	(0.70)
Letter cancellation single (seconds)	62.95	(23.24)	58.77	(11.31)
Errors	0.37	(1.10)	0.05	(0.21)
Letter cancellation double (seconds)	119.89	(33.70)	102.68	(18.55)
Errors	1.05	(1.78)	0.82	(1.30)
Trial-making A (seconds)	31.95	(11.36)	28.64	(9.76)
Trail-making B (seconds)	93.00	(52.69)	60.82	(23.44)
Self-report measures				
BDI	10.74	(10.01)	3.15[a]	(4.17)
STAI				
State	45.14[b]	(11.51)	29.58[c]	(6.32)
Trait	44.47	(10.08)	33.25[d]	(9.26)
LEYTON				
State	16.84	(11.26)	9.43[c]	(9.21)
Trait	9.26	(4.75)	5.30[a]	(2.98)

GTS, Gilles de la Tourette syndrome; PASAT, paced and unpaced auditory serial addition test; BDI, Beck Depression Inventory; STAI, State-Trait Anxiety Inventory; LEYTON, Leyton Obsessional Inventory.

[a] $N = 20$.
[b] $N = 14$.
[c] $N = 12$.
[d] $N = 16$.
[e] $N = 21$.

serial addition tasks. Within the GTS group, there were significant negative correlations between IQ and number of errors on unpaced ($r = -0.59$, $p < 0.01$) and 2-second serial addition ($r = -0.63$, $p < 0.01$); there were no significant correlations within the normal control group.

Block Sequence Span

Repeated measures analysis of variance was performed to compare the groups on the two tasks, forward and backward span, with one between-subjects factor (group) and one within-subjects factor (task). The group × task interaction approached significance ($F = 3.17$, $df = 1,39$, $p < 0.09$), and there was a significant effect of group ($F = 4.59$, $df = 1,39$, $p < 0.04$). Post-hoc t tests showed a significant group difference in forward span ($t = 2.74$, $df = 39$, $p < 0.01$), but the difference in backward span did not reach significance. Mean scores showed that the control group tended to recall forward sequences slightly better than backward sequences, whereas the GTS subjects tended to perform at the same level both forwards and backwards. There were no significant correlations within each group between performance on the tasks and age or sex, although IQ correlated positively with backward span for the GTS group ($r = 0.55$, $p < 0.01$).

Stroop Test

Repeated measures analysis of variance was performed to compare the groups on the two tasks, control Stroop and conflicting colour Stroop, with one between-subjects factor (group) and one within-sub-

jects factor (task). The group × task interaction did not reach significance, nor was there a significant effect of group. Errors were rare, and chi-squared tests revealed no significant differences between the groups in the number of errors made in each task.

Letter Cancellation Test

Repeated measures analysis of variance was performed to compare the groups on the two tasks, single-letter cancellation and double-letter cancellation, with one between-subjects factor (group) and one within-subjects factor (task). There was a significant group × task interaction ($F = 5.99$, $df = 1,39$, $p < 0.02$). Post-hoc t tests showed that the groups did not differ significantly on the single-letter task; the difference neared significance on the double-letter task ($t = 2.06$, $df = 1,39$, $P < 0.05$), with the GTS group tending to perform slower than the controls. Errors were infrequent, and chi-squared tests showed that the groups did not differ significantly in error rates on each task. There were no significant correlations with sex, age, or IQ within the GTS group; age correlated significantly with speed of performance on both single- ($r = 0.54$, $p < 0.01$) and double-letter cancellation ($r = 0.65$, $p < 0.01$) within the normal control group.

Trail-making Test

Repeated measures analysis of variance was performed to compare the groups on the two tasks, A (ascending numbers alone) and B (alternating ascending numbers and letters), with one between-subjects factor (group) and one within-subjects factor (task). There was a significant group × task interaction ($F = 6.72$, $df = 1,39$, $p < 0.02$). Post-hoc t tests revealed that the groups did not differ significantly on A, but there was a significant difference on B ($t = 2.59$, $df = 39$, $p < 0.02$), showing the GTS group to perform slower than the controls on this task. Within the GTS group, age correlated significantly with speed of performance on A ($r = 0.55$, $p < 0.01$), but there were no other significant correlations with sex, age, or IQ; nor did these correlate significantly with performance for the normal control group.

Effects of Medication

Since a proportion of the GTS group were taking dopamine antagonists or serotonin reuptake inhibitors, the relationships between medication and results on the experimental tasks were examined. In order to equate levels of drug, the minimum therapeutic dose recommended by the British National Formulary (25) for each individual drug was taken to represent one unit, and doses prescribed to each individual patient were represented in units as multiples of the minimum therapeutic dose. Analyses were also performed using presence or absence of medication as the defining feature. None of the correlations between performance on the experimental tasks and usage of dopamine antagonists or serotonin reuptake inhibitors reached significance.

Effects of Psychopathology

Mean scores and standard deviations for the self-report measures of depression, anxiety, and obsessionality for the two groups are reported in Table 1. The groups differed significantly in level of depressed mood ($t = 3.02$, $df = 37$, $p < 0.01$). Using repeated measures analysis of variance with one between-subjects factor (group) and one within-subjects factor (state/trait), the group × state/trait interaction was not significant, but there was a significant group effect for anxiety ($F = 17.73$, $df = 1,24$, $p < 0.01$). Post-hoc t tests revealed a significant group difference in both state ($t = 4.17$, $df = 24$, $p < 0.01$) and trait anxiety ($t = 3.41$, $df = 33$, $p < 0.01$). Repeated measures analysis of variance for obsessionality with one between-subjects factor (group) and one within-subjects factor (state/trait) also showed a nonsignificant group × state/trait interaction, and a significant group effect ($F = 6.32$, $df = 1,37$, $p < 0.02$). The t tests revealed a near-significant group difference in state obsessionality ($t = 2.29$, $df = 38$, $p < 0.03$) and a significant difference in trait obsessionality ($t = 3.14$, $df = 37$, $p < 0.01$). For each of the self-report measures, the mean score for the GTS group was higher than for the control group.

In order to examine any relationship between the self-report measures and performance on the experimental measures that differentiated the two groups, Pearson product moment correlations were calculated within each group. Performance on the letter cancellation and trail-making tasks did not correlate significantly with depressed mood, trait anxiety, state, or trait obsessionality within each group. Within the normal control group, there was a significant association between state anxiety and performance on trail-making A (letters only) ($r = 0.88$, $p < 0.01$), although only half the sample had completed this measure due to an administrative error. There were no other significant correlations with state anxiety for either group.

DISCUSSION

The main findings were that the GTS subjects were significantly impaired relative to normal controls on several of the experimental tasks: serial addition, block sequence span (forwards), joining ascending sequences and cancelling target letters, particularly when alternation between two sets of targets was required in the latter two tasks. Since the groups did not differ significantly in IQ, which was in the normal range, these findings represented selective deficits rather than global impairments in functioning for the GTS group.

Serial addition taps working memory by requiring subjects to hold two numbers in mind while performing addition on serial pairs of these, and the GTS subjects were impaired relative to controls on each of the three tasks. Errors might be produced by difficulties in sustaining attention, by loss of information from working memory of either the numbers themselves or their temporal order, by arithmetic mistakes, or by forgetting of the correct rules. Full error analysis was not possible, since failures to respond could signify several different types of error, and a more detailed qualitative study would be needed to pin these down precisely. The significant negative association between IQ and error rates within the GTS group on two of the three tasks suggests general ability to be a contributory factor, but this did not greatly influence performance in the control group.

Block sequence span is primarily a nonverbal working memory task. The forward task involves retaining a spatial sequence long enough to reproduce it correctly; the backward task also requires retention of the sequence, and mental reversal of its order before reproduction. The GTS subjects failed to show the normal advantage on the forward task, but performed similarly to the control subjects on reversed sequences. Again, general ability appeared to influence performance on reversed sequences for the GTS group, but not for the control group.

In both the trail-making and letter cancellation tests, the GTS group showed relatively greater difficulty compared to controls on the more complex task for each test. The second of the trail-making tasks involves tracking two ascending sequences (letters and numbers) in working memory and alternating between these, making a visual search for the next target, and making a motor response to join the targets. The second letter cancellation task also involves holding two target letters in working memory while making a serial visual search to identify appropriate targets among distractor letters, and making a motor response to cross out targets. The GTS subjects were significantly slower than controls under these conditions. Interestingly, they did not make significantly more errors than the control group in either of the letter cancellation tasks, suggesting slowing of performance to be a successful strategy allowing maintenance of an accurate response. In both trail-making and letter cancellation, the GTS subjects did not differ significantly from the controls in the first task, which involved only a single sequence of letters or a single target letter, respectively. Since both the first and second tasks in both trail-making and letter cancellation involved similar visual search and motor responses, this suggests that their difficulties on the second task in each test are unlikely to be attributable to visual search deficits or slow motor speed alone. Impairment is more likely to be attributable to difficulties in sustaining attention, holding information in working memory, or shifting set appropriately between two sets of targets. Unimpaired performance on the Stroop test provides further support for the view that slow speed of performance (verbal rather than motor in this test) is not an adequate explanation of deficits in the GTS group.

Speed on certain tasks did show some relationship with age, and IQ influenced performance significantly within the GTS group on others. However, the lack of significant correlations between most of the attentional measures and sex, age, or IQ within each group suggests that these did not play an important role in determining performance. There was also no evidence in our study that medication played an important role in producing effects on cognitive function. This is consistent with the conclusion reached by Golden (6) on the basis of a review of the literature on neuropsychological function in GTS, that deficits found appeared to be related to the underlying condition rather than to medication per se. Nevertheless, the possibility remains that attentional deficits of this nature could be attributable to medication, and more studies will be necessary to investigate this possibility further.

The standard deviations of the GTS group on the experimental measures were generally larger than those of the control group, indicating greater variability in performance. Intercorrelations between the experimental measures were performed for the GTS group (can be supplied by the authors on request), and these were often significant, suggesting that individuals tended to perform consistently across tests. This could be related to other reports of heterogeneity in the clinical features of GTS (2, 26, 27). However, in

view of the small sample sizes, the results should be viewed with caution.

The GTS subjects did report significantly greater depressed mood, anxiety, and obsessional symptoms relative to the controls. There is evidence from other areas that certain aspects of cognitive performance are adversely affected by emotional disorders (see ref. 28 for a review). One potential explanation for this is that the amount of cognitive capacity available to process information is reduced because some capacity is taken up by task-irrelevant processing. Ellis and Ashbrook (29) have postulated that, during depressive illness, depressive ruminations reduce available processing capacity. Eysenck (30) put forward an account of cognitive deficits in anxiety in terms of capacity limitations produced by extraneous processing of anxious thoughts. However, if emotional factors largely account for such a reduced capacity, performance deficits might reasonably be expected to increase with higher levels of emotional disturbance. The lack of significant correlations between the attentional measures and the self-report questionnaires for each group in almost every case does not lend strong support to this argument. An alternative explanation might be found in relation to GTS symptoms, such as motor tics and vocalisations. These are thought to be largely involuntary, and a range of neurophysiological evidence has been cited to support this view (31), although there is a minority who suggest the movements are intentional (32). A reduction in available cognitive resources might be produced by the occurrence of the symptoms themselves, distracting attention and thereby disrupting performance; by attempts to suppress symptoms consciously; or by distracting thoughts such as social embarrassment centred round the occurrence of symptoms. However, it would still be necessary to account for the particular pattern of deficits shown.

The findings do support clinical descriptions and reports of attentional deficits in GTS. Impairment was found both in sustaining attention, and in focusing and shifting set between salient stimuli. Further work is needed in order to explore the nature of the deficits, and to investigate possible underlying mechanisms involved, and the relevant brain pathophysiology. As the frontal cortex has been implicated as having a role in attention (33), and it has been suggested that frontal-subcortical systems may be involved in the pathophysiology of GTS (34–36), our findings of deficits in attention in GTS are particularly important.

Greater understanding of attentional deficits in GTS has important implications for everyday life, such as in guiding means of adapting teaching methods to maximise learning.

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Executive Function, Memory, and Learning in Tourette's Syndrome

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Young people with Tourette's syndrome (TS) alone, TS plus attention-deficit/hyperactivity disorder (+ADHD), or TS plus obsessive–compulsive disorder (+OCD) were compared with a healthy control group on a set of measures of executive functioning, memory, and learning. The TS-alone group was impaired on one executive measure involving inhibition and strategy generation but did not differ significantly from the healthy control group on other measures. The TS+ADHD group showed impairment on several executive measures. There was no evidence of impairment in implicit aspects of memory and learning for any of the TS groups. The findings are discussed in terms of the frontostriatal hypothesis of TS and the contribution of comorbid symptomatology.

Tourette's syndrome (TS) is a neurodevelopmental disorder in which the core symptomatology consists of motor and vocal tics. A range of cognitive and behavioral features is often described in conjunction with the tics. TS has been linked to a range of everyday problems suggestive of inhibitory deficits including coprolalia and copropraxia, social inappropriateness (Kurlan et al., 1996), "disinhibited behaviors" (Cohen & Leckman, 1992), and criminal behavior (Kurlan et al., 1996). Early cognitive studies of TS (e.g., Channon, Flynn, & Robertson, 1992) suggested it to be associated with impairment in aspects of executive functioning. However, these early studies failed to take an adequate account of comorbid symptomatology, and controversy exists as to whether any impairments in executive functioning are directly attributable to TS itself or to comorbid features, in particular attention-deficit/hyperactivity disorder (ADHD) or obsessive–compulsive disorder (OCD). Pennington and Ozonoff (1996) postulated that cognitive functioning in TS might be associated primarily with selective inhibitory dysfunction because it is characterized by difficulties in suppressing unwanted motor movements and vocalizations.

Studies investigating the brain basis of TS have commonly postulated that frontostriatal pathways are compromised (see e.g., Chase, Geoffrey, Gillespie, & Burrows, 1986; Moriarty et al., 1997; Robertson, 2000). Prevailing theory has implicated dopaminergic basal ganglia circuitry in the etiology of TS, with potential disruption of frontosubcortical circuits involving nonoverlapping parts of the striatum, globus pallidus, substantia nigra, thalamus, and frontal cortex (Alexander, DeLong, & Strick, 1986), although other researchers have suggested alternative models (see e.g., Weeks, Turjanski, & Brooks, 1996). Support for the theory of basal ganglia dysfunction comes in large part from the relative efficacy of dopamine antagonists in treating TS symptoms (Robertson, 2000); eye movement studies, imaging, and biochemical data have provided additional, although sometimes conflicting, evidence. Both functional and structural imaging have suggested abnormalities in the basal ganglia, most commonly the caudate (e.g., Hyde et al., 1995; Malison et al., 1995; Moriarty et al., 1997); direct involvement of frontal regions including the anterior cingulate cortex has also been described in an early positron emission tomography study (Chase et al., 1986), although these studies have often failed to assess comorbid symptomatology. Recent functional imaging studies of normal participants have suggested that the anterior cingulate plays a central role in inhibitory processes by detecting potential response competition in tasks requiring inhibition of a competing prepotent response (e.g., Carter et al., 1998).

The Stroop Color–Word Test (Stroop, 1935) has most commonly been used to study inhibitory functioning in TS. Pennington and Ozonoff (1996) concluded from a review of performance on inhibitory tasks that there was inconsistent evidence of impairment in TS, perhaps because of comorbidity issues. There is little to suggest impairment in TS participants on aspects of executive function thought to involve dorsolateral frontal areas including planning, rule finding, and set shifting (Bornstein, 1991b; Ozonoff & Jensen, 1999) using tasks such as the Wisconsin Card Sorting Test (WCST; Heaton, 1981). However, lateral frontal areas are thought to contribute to strategic aspects of memory encoding and retrieval, and impairment on tasks including recall of categorized words and listening memory span thought to involve strategic, explicit aspects of memory has been described in TS alone (Stebbins et al., 1995). Little work has been done examining implicit aspects of memory

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and learning in TS, although impaired implicit skill learning has been reported in one study using a rotary pursuit task (Stebbins et al., 1995).

Although previous studies have addressed these issues, very little work has been done that compared groups of people who have TS with and without comorbid symptomatology using a comprehensive range of tests involving both inhibitory and other aspects of executive function, explicit and implicit memory, and learning. To separate the effects of TS from those of comorbid symptomatology, we compared a subgroup who had TS alone with participants who had TS plus ADHD (TS+ADHD), TS plus OCD (TS+OCD), and a matched healthy control group. It was predicted that all TS groups might show impairment on executive tasks involving inhibitory control and that comorbid symptomatology might be linked to more severe and possibly more widespread deficits.

Method

Participants

Twenty-nine participants (19 male, 10 female) who met the *Diagnostic Statistical Manual of Mental Disorders* (4th ed., text rev.; *DSM–IV–TR*; American Psychiatric Association, 2000) criteria for TS took part in the study. To establish the diagnosis and rule out any comorbid disorders other than ADHD or OCD, we assessed participants using the "National Hospital Interview Schedule for Gilles de la Tourette Syndrome" (Robertson & Eapen, 1996). Participants who met *DSM–IV–TR* criteria for a comorbid psychiatric disorder other than ADHD or OCD (e.g., psychosis) were excluded from the study, as were participants with any history of neurological disorder (e.g., head injury). Inclusion criteria were fluency in English and age between 9 and 18 years. Fourteen of the TS participants were diagnosed with TS alone, 9 with TS+ADHD, and 6 with TS+OCD. Fourteen (48.3%) of the TS participants were taking medication at the time of testing, comprising 7 (50.0%) of the TS-alone group, 3 (33.3%) of the TS+ADHD group, and 4 (66.7%) of the TS+OCD group. Eight participants were taking antipsychotic preparations: 5 were taking antipsychotic preparations alone, 2 were taking antipsychotic preparations in combination with selective serotonin reuptake inhibitors (SSRIs), and 1 was taking an antipsychotic preparation in combination with a stimulant. Two participants were taking SSRIs alone, 2 were taking an antisympathetic drug alone, 1 was taking a tricyclic drug alone, and 1 was taking a stimulant drug alone.

Twenty-one matched healthy control participants (13 male, 8 female) also took part in the study. The control group and TS subgroups were compared using an analysis of variance (ANOVA), and this showed that they did not differ significantly in age, $F(3, 46) = 1.79$, $p = .163$, or on Raven's standard progressive matrices (prorated; Raven, 1960), $F(3, 46) = 0.97$, $p = .416$. Mean scores and standard deviations are shown in Table 1. Written informed consent was given by parents or guardians for all participants. Breaks were given between tasks as necessary to avoid fatigue.

In addition to the clinical ratings described above, several symptomatology measures were also used. First, tic severity was assessed using the Yale Global Tic Severity Scale (Leckman et al., 1989); no significant differences were found between the three TS groups, $F(2, 25) = 0.20$, $p = .818$. Second, the groups differed as expected when compared on ADHD and OCD symptomatology measures, namely the Brown Attention-Deficit Disorder Scales (Brown, 1996) and the short Leyton Obsessional Inventory (Berg, Whitaker, Davies, Flament, & Rapoport, 1988). For the Brown Attention-Deficit Disorder Scale, ANOVA showed a significant group effect, $F(3, 43) = 7.61$, $p = .0001$, and the TS+ADHD group scored higher than all the other groups. Scheffé tests confirmed that this difference reached significance for comparison with the control group ($p = .0001$). For the Leyton Obssessional Inventory, ANOVA showed a significant group effect, $F(3, 43) = 13.92$, $p = .0001$, and the TS+OCD group scored higher than all the other groups. Scheffé tests confirmed that this difference reached significance for comparison with the control group ($p = .0001$), the TS-alone group ($p = .0001$), and the TS+ADHD group ($p = .001$). Third, the parent form of the Child Behavior Checklist (Achenbach, 1991) was also administered, and this showed a significant effect of group, $F(3, 43) = 13.24$, $p = .0001$. Scheffé tests showed that the TS+ADHD group scored significantly higher than the control group ($p = .0001$) and the TS-alone group ($p = .004$); the TS+OCD group, in turn, scored significantly higher than the control group ($p = .041$).

Executive Measures

Tests were selected to assess aspects of executive function. The executive functions tested were as follows.

Inhibition and strategy generation. First, the Hayling Test (Burgess & Shallice, 1997) involved completing sentences as

Table 1
Mean Scores and Standard Deviations for the Demographic and Clinical Symptomatology Measures

	Group								
	TS alone ($n = 14$)		TS+ADHD ($n = 9$)		TS+OCD ($n = 6$)		Control ($n = 21$)		
Variable	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*p*
Age (years)	13.71	2.05	12.33	2.24	15.17	2.48	13.67	2.52	> .05
Raven's matrices	40.50	9.49	38.00	9.29	35.50	7.61	41.33	6.92	> .05
Yale tic severity scale[a]	32.64	20.98	27.88	21.64	27.83	13.29			> .05
Brown attention deficit scale	39.43	22.06	60.00	16.37	46.33	20.01	25.76	14.03	.0001
Leyton Obsessional Inventory	4.50	6.43	5.22	6.00	17.17	5.27	2.10	3.05	.0001
CBCL[a,b,c]	39.85	17.47	76.25	28.94	52.00	24.65	22.90	17.89	.0001

Note. TS = Tourette's syndrome; +ADHD = plus attention-deficit/hyperactivity disorder; +OCD = plus obsessive–compulsive disorder; CBCL = Child Behavior Checklist.
[a] $n = 8$ in the TS+ADHD group. [b] $n = 13$ in the TS alone group. [c] $n = 20$ in the control group.

quickly as possible with words that make sense (Part A) and with nonsensical words (Part B). Patients with frontal-lobe lesions have been found to have difficulty both in simple response initiation (Part A) and in inhibiting straightforward completion words to generate nonsensical completion words (Part B). For Part B, the number of errors involving directly related completion words (Category A errors) was scored, and also the number of errors involving related but less direct completions (Category B errors) was scored. Second, the Stroop test involved naming the color of ink in which words spelling out colors are written (e.g., *red* is written in green ink). Third, the Letter Fluency Test (Thurstone & Thurstone, 1962) required the generation of as many words beginning with *s* as possible in 5 min.

Multitasking. The Six Elements Test (SET; Wilson, Alderman, Burgess, Emslie, & Evans, 1996) involved carrying out a set of six tasks within a 10-min period with certain rule constraints about the order in which the tasks should be performed. The task instructions informed participants that the tasks could not all be completed within the time limit and that they would score better by attempting items from each of the tasks rather than by completing a small number. No cues were given to assist the participants in planning the order of tasks attempted or in monitoring the passage of time other than providing them with a clock. Patients with frontal-lobe lesions have been shown to have difficulties in electing to alter their behavior appropriately in the absence of environmental cues to maximize gains by attempting fewer tasks or by breaking the rules more frequently than comparison groups. The score obtained for the test takes into account both the allocation of time to the different tasks and the order of performance.

Rule following and set shifting. First, the Rule Shift test (Wilson et al., 1996) involved responding as quickly as possible to a series of playing cards according to one rule ("yes" to red, "no" to black), and then shifting to a different rule ("yes" to cards with the same color as the previous one, "no" to cards of a different color) with a second series of cards. This test, therefore, contained a rule-shifting component but was simpler than tests such as the WCST because the Rule Shift test did not involve deductive reasoning to work out which rule was in operation. Normative data are provided in the manual for healthy control adults and for those with neurological lesions. The score obtained for the test takes account of the number of errors made and the time taken. Second, the Trail Making Test (Reitan, 1958) required sequencing by number (Part A) and alternation between number and letter (Part B).

Memory and Learning Measures

Tests were also selected to assess explicit and implicit aspects of memory and learning. These tests were as follows.

Explicit. First, the Adult Memory and Information Processing Battery (AMIPB) story recall (Coughlan & Hollows, 1985) involved listening to a story, repeating it immediately, and recalling it after a filled time delay. Second, the Rey Auditory Verbal Learning Test (RAVLT; Rey, 1964) involved learning a 15-word list over five trials, an interfering list, and a final recall. Third, the Visual Reproduction test of the Wechsler Memory Scale—Revised (WMS−R; Wechsler, 1987) required studying simple geometric drawings, drawing them immediately, and remembering them after a filled time delay.

Implicit. First, priming (based on Warrington & Weiskrantz, 1974) was assessed after a study phase in which participants made liking judgments for 40 low-frequency words, half presented in a male voice and half in a female voice. Priming effects were measured by presenting 40 word stems aurally (e.g., "clo") and asking participants to give the first word that came to mind. Each of the three-letter word stems could be completed to form at least 10 common English nouns. Twenty of the word stems could be used to complete words presented in the initial phase, and 20 were new distractor items taken from target words matched in frequency and number of alternative word endings to the study words. Learning was measured by comparing the number of primed words given (out of 20) versus the number of times the distractor word given (out of 20) was a target word of the same frequency as the primed words. Second, skill acquisition (based on Cohen & Squire, 1980) was examined by presenting participants with mirror-reversed words and asking the participants to read the words aloud as quickly as possible. All words were low-frequency nouns, between five and eight letters. After a practice trial, 20 triads of words were presented. After a 30-min filled delay, 10 of the study triads were presented, mixed with 10 new triads. Learning was measured by the decrease in the time taken to complete the second triad compared with the first. Third, in the Serial Reaction Time (SRT) task (based on Nissen & Bullemer, 1987), learning was assessed by presenting a series of letters on a computer screen in four different spatial locations and asking participants to select the matching spatial response as quickly as possible. The screen display consisted of a central box and four boxes arranged in a cross, and the response apparatus consisted of four response keys and a central home key corresponding to the spatial locations on the screen. Each response key was labeled *U* for up, *D* for down, *L* for left, and *R* for right. Each trial started when the participant pressed the central home key, which was signaled by a central box on the screen lighting up. For each trial, a letter appropriate to the location appeared in one of these four boxes (e.g., *U* in the upper box), and participants had to press the corresponding key on the response apparatus. Letters were presented 100 ms after the central home key was pressed and terminated with the response. Each condition consisted of eight blocks of 120-letter presentations with a 30-s break between each block of trials. Unknown to participants, these at times appeared in a repeating sequence. Two pseudorandom practice blocks were given initially to eliminate nonspecific effects; the next four blocks contained a 12-item sequence, *ULDUDRLURDLR*, followed by a different test sequence, *RULRDURLDRUD*, and finally another block of the original sequence. Overall, reaction time (RT) decrease with sequence learning was assessed by comparing median RT scores for Block 6 divided by Block 3; sensitivity to the specific sequence was assessed by comparing RT slowing on Block 7 divided by Block 8.

Results

The groups were compared using ANOVA, with Scheffé tests for post hoc comparisons as appropriate. Level of significance was adjusted for each set of measures by dividing .05 by the number of measures. Logarithmic transformations were used for several variables (Hayling times, Trail Making times, Stroop scores).

Mean scores and standard deviations for the tests of executive functions are shown in Table 2. Mean scores and standard deviations for the tests of memory and learning are shown in Table 3.

Executive Measures

Inhibition and strategy generation. Because four measures of inhibition and strategy generation were used, a significance level of .05/4 (.0125) was adopted. On the Hayling test, ANOVA showed that the groups differed

Table 2
Mean Scores and Standard Deviations for the Tests of Executive Function

	Group								
	TS alone (n = 14)		TS+ADHD (n = 9)		TS+OCD (n = 6)		Control (n = 21)		
Variable	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*p*
Inhibition and strategy generation									
Hayling test									
Category A errors	1.43	1.16	2.11	1.54	0.50	0.84	0.38	0.67	.0001
Category B errors	2.50	1.99	3.78	2.44	2.83	2.14	1.24	1.22	.007
Stroop test	77.50	25.95	62.44	21.09	64.50	20.38	81.67	25.94	> .050
Letter Fluency	27.79	11.18	24.11	6.57	24.50	7.20	33.33	9.26	.044
Multitasking									
Six Elements Test	2.57	1.40	1.67	1.12	2.50	1.22	3.33	1.02	.008
Rule following/ set shifting									
Rule Shift test	3.21	0.98	3.00	1.22	3.67	0.52	3.57	0.51	> .050
Trail Making Test									
Part A (s)	35.69	22.14	31.24	10.84	43.61	15.36	28.29	9.82	> .050
Part B (s)	79.85	33.83	91.15	43.44	92.71	26.17	67.99	26.98	> .050

Note. TS = Tourette's syndrome; +ADHD = plus attention-deficit/hyperactivity disorder; +OCD = plus obsessive–compulsive disorder.

significantly in both the number of Category A errors, $F(3, 46) = 7.30$, $p = .0001$, and the number of Category B errors, $F(3, 46) = 4.59$, $p = .007$. Scheffé comparisons of individual groups revealed that the TS-alone group made significantly more Type A errors than the control group ($p = .045$), and the TS-alone group neither differed from the control group for Type B errors nor differed from the other TS groups. The TS+ADHD group made more Type A and B errors than the other groups, differing significantly from the control group for both A ($p = .002$) and B ($p = .012$) errors and from the TS+OCD group for A ($p = .043$) errors. On the Stroop task, the effect of group was not significant, $F(3, 46) = 1.71$, $p = .179$. The effect of group was also not significant on Letter Fluency, using a strict significance criterion, $F(3, 46) = 2.92$, $p = .044$.

Multitasking. Scores for the SET showed a significant effect of group, $F(3, 46) = 3.80$, $p = .008$. Scheffé tests confirmed that the TS+ADHD group scored significantly below the control group ($p = .010$); no other group differences were significant.

Table 3
Mean Scores and Standard Deviations for the Tests of Memory and Learning

	Group								
	TS alone (n = 14)		TS+ADHD (n = 9)		TS+OCD (n = 6)		Control (n = 21)		
Variable	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*p*
Explicit									
AMIPB delayed story recall	34.07	9.19	38.22	7.71	26.33	10.89	34.33	8.46	> .050
Delayed Visual Rep.	34.29	6.18	27.44	7.07	29.50	10.09	34.33	4.87	.030
RAVLT									
Total Trials 1–5	49.93	11.15	46.56	5.15	48.17	9.93	55.24	6.24	.039
Trial 6	11.43	3.59	9.00	2.40	10.00	2.68	12.19	2.62	.044
Implicit									
Stem completion	2.00	2.63	1.11	2.03	2.33	1.99	1.57	2.34	> .050
Mirror reading	0.73	0.11	0.73	0.11	0.66	0.08	0.64	0.11	> .050
SRT[a]									
RT Block 6/Block 3	0.90	0.07	0.91	0.10	0.90	0.06	0.89	0.11	> .050
RT Block 7/Block 8	1.06	0.08	1.08	0.10	1.04	0.03	1.10	0.15	> .050

Note. TS = Tourette's syndrome; +ADHD = plus attention-deficit/hyperactivity disorder; +OCD = plus obsessive–compulsive disorder; AMIPB = Adult Memory and Information Processing Battery; Visual Rep. = Visual Reproduction test of the Wechsler Memory Scale—Revised; RAVLT = Rey Auditory Verbal Learning Test; SRT = Serial Reaction Time task; RT = reaction time.
[a] $n = 8$ in the TS+ADHD group.

Rule following and set shifting. A significance level of .05/3 (.0167) was used. The effect of group was not significant for the Rule Shift test, $F(3, 46) = 1.47$, $p = .236$. There was also no significant group effect for the Trail Making Test Part A, $F(3, 46) = 2.27$, $p = .094$, or Part B, $F(3, 46) = 1.80$, $p = .161$.

Spearman rank correlations with the symptomatology measures for each group were examined separately for the three executive measures that significantly discriminated between the groups. There were no significant correlations for the control group using a significance level of .05. For the TS-alone group, the number of Hayling Category A errors correlated significantly with Brown attention-deficit scores, $r_s = .54$, $p = .048$. For the TS+ADHD group, the number of Hayling Category A errors correlated significantly with the Child Behavior Checklist, $r_s = .77$, $p = .024$. For the TS+OCD group, the number of Hayling Category A errors correlated significantly with Yale Tic Severity scores, $r_s = -.85$, $p = .034$. These correlations were in the expected direction, except for the TS+OCD correlation.

For the TS-alone group, the number of Hayling Category A errors was the only measure that significantly differentiated them from the control group. Because the Hayling Category A errors also correlated significantly with ADHD scores for the TS-alone group, an analysis of covariance (ANCOVA) was performed to compare these two groups using the Brown attention-deficit score as a covariate. The effect of group remained significant ($p = .013$).

Memory and Learning Measures

Explicit. A significance level of .05/4 (.0125) was used. ANOVA for delayed AMIPB story recall showed no significant effect of group, $F(3, 46) = 2.20$, $p = .101$. For the Visual Reproduction test, the effect of group did not reach significance using a strict criterion, $F(3, 46) = 3.25$, $p = .030$. On the RAVLT, the effect of group did not reach significance using a strict criterion for number of words recalled over the learning trials, $F(3, 46) = 3.03$, $p = .039$, or number of words recalled after an interfering list, $F(3, 46) = 2.91$, $p = .044$.

Implicit. A significance level of .05/4 (.0125) was used. For the priming task, there was no significant group difference for the number of primed-target distractor words, $F(3, 46) = 0.44$, $p = .729$. For the mirror reading task, ANOVA did not show a significant group effect for speeding up on Trial 2 divided by Trial 1, $F(3, 46) = 2.24$, $p = .096$. For the SRT task, there was no significant group difference in speed of sequence learning when Block 6 divided by Block 3 was compared, $F(3, 45) = 0.14$, $p = .934$. There was also no significant group difference in RT slowing on the transfer test when Block 7 divided by Block 8 was examined, $F(3, 45) = 0.63$, $p = .602$.

Effects of Medication and Age

The effects of medication on performance were examined in relation to the three executive measures that had significantly differentiated the groups. There were no significant differences between the medicated and the unmedicated participants except for the SET scores for the TS+OCD group in which the unmedicated participants scored lower, $t(4) = 6.00$, $p = .004$.

Although the groups did not differ significantly in age, the TS+OCD participants were the oldest in mean age, and the TS+ADHD participants were the youngest. Although older age in the TS+OCD group does not seem to be a probable explanation for impairments on some tasks, younger age in the TS+ADHD group is a potentially relevant factor, because they also showed the greatest evidence of impairment. To examine any effects of age, we compared the three executive measures that had significantly differentiated the groups using ANCOVA with age as a covariate. This showed that the effect of group remained significant for each of the measures.

Discussion

The present study explored cognitive functioning in TS using a wide range of tests and using careful screening to identify those with TS alone and those with comorbid ADHD or OCD symptomatology. For the executive measures, there was a significant effect of group on two of the inhibitory measures (Hayling Category A and B errors) and the multitasking measure (SET), and the group effect approached significance using a strict criterion on the Letter Fluency measure. There was no evidence of impairment associated with TS in implicit aspects of memory and learning, although the group differences approached significance for several of the explicit memory measures (Visual Reproduction, RAVLT learning, and RAVLT recall after an interfering list). Post hoc comparison of the groups for the three executive measures that differentiated them significantly showed that the TS+ADHD group differed significantly from the control group on each of these three measures, the TS-alone group differed significantly from the control group on one measure (Hayling Category A), and the TS+OCD group did not differ significantly from the control group on any of the measures.

Although the groups did not differ significantly in age, the TS+ADHD group was the youngest. The effects of age were therefore explored, but this did not alter the findings. Severity of tic symptomatology did not appear to explain differences between the TS groups because they were similar on the Yale tic severity measure. Although some of the TS participants in each group were on medication, there was little to suggest that this was an important factor in determining the pattern of findings. Small sample size may have been an issue in relation to the failure to detect differences on other tasks between the TS-alone and the control groups because several of the measures failed to reach significance using a strict criterion to adjust for multiple comparisons. This may be another factor contributing to the inconsistent evidence with respect to inhibitory impairment in TS alone in the existing literature, especially if any associated deficits are relatively mild. Nevertheless, differences between the groups emerged clearly on several of the executive mea-

sures despite the small group sizes, especially for the TS+ADHD and TS+OCD samples. Taken together, the present findings suggest that comorbid ADHD symptomatology is the biggest contributor to any deficits associated with TS.

With respect to TS alone, previous studies have suggested that any cognitive deficits may be confined to restricted aspects of executive functioning involving inhibitory processes. The present study provided some support for this. There was no evidence of impairment on the Trail Making Test or the Rule Shift test, adding weight to the conclusion that these aspects of performance are intact in TS alone. The Rule Shift test used in the present study was a less demanding task than the WCST and, therefore, may have been less sensitive to possible differences. However, this seems an improbable explanation given the lack of differences found on the WCST by other groups. The WCST has been found to be intact in both adults and children with mixed TS and in people with TS alone and TS+OCD (Bornstein, 1991b; Channon, Crawford, Vakili, & Robertson, 2003; Ozonoff & Jensen, 1999; Schuerholz et al., 1996). Bornstein (1991a) found that children who had TS with high versus low OC symptoms performed worse on the WCST; no control group was included. Similarly, there is little evidence of difficulties in uncomplicated TS on other executive tasks involving planning and set shifting. For instance, intact planning performance using the Tower of Hanoi has been reported in children with mixed TS (Ozonoff & Jensen, 1999). Impairment on Part B of the Trail Making Test has been reported in some adults with mixed TS (e.g., Channon et al., 1992) but not in others (e.g., Bornstein, 1991b). OC symptomatology did not appear to contribute to performance deficits in the Channon et al. (1992) study, but ADHD symptomatology was not assessed. Comorbid symptomatology may potentially have adverse effects, as indicated by the impairment found in the TS+ADHD group on the multitasking test.

Although relatively little work has been done examining issues related to memory and learning in TS, there is some evidence of difficulties in strategic aspects of memory functioning that involve explicit learning of the relevant information. The most comprehensive study to date was carried out by Stebbins et al. (1995), who reported impairment in a group of adults with TS alone on tasks including recall of categorized words and listening memory span. Channon et al. (2003) used a story recall task and also found impairment in a group of adults with TS alone. The present study did not find evidence of impairment in either explicit or implicit memory and learning in TS alone, although the effect of group did approach significance for several of the explicit measures, and small sample sizes may therefore have led to an underestimate of any difficulties on these. Mean scores show that the scores of the TS-alone group were closest to those of the control group and that those with comorbid ADHD or OCD diagnoses tended to perform lower. Further work is needed to examine explicit memory performance in those with TS alone. There is also little work exploring the issue of implicit memory and learning, although impairment has been reported in relation to motor skill learning on the pursuit rotor task (Stebbins et al., 1995). Several different implicit learning tasks thought to have different brain bases were used in the present study, and there was no evidence of difficulties either in the TS-alone group or in those with comorbid diagnoses on any of the measures.

By contrast, the TS-alone group in the present study did show clear evidence of impairment on an inhibitory task, the Hayling test. Although performance on this measure for the TS-alone group correlated significantly with ADHD symptomatology, ANCOVA using ADHD scores as the covariate showed that this finding was robust because the TS-alone group still scored below the level of the control group. Impairment on the Hayling test has also been found in adults with TS alone (Channon et al., 2003). Imaging studies examining inhibitory performance on the Hayling test have shown activation in normal participants in the right anterior cingulate and left frontal operculum (Nathaniel-James, Fletcher, & Frith, 1997), the left inferior frontal gyrus (Nathaniel-James et al., 1997; Collette et al., 2001), and the left middle frontal gyrus (Collette et al., 2001). This appears broadly consistent with the prevailing view that TS is associated with basal ganglia abnormalities leading to disruption of dopaminergic fronto-subcortical circuits, although unequivocal evidence regarding the brain basis of TS is currently lacking (see, e.g., Robertson, 2000). Multiple fronto-subcortical circuits have been described involving nonoverlapping parts of the striatum, globus pallidus, substantia nigra, thalamus, and frontal cortex (e.g., Alexander et al., 1986). Greater specificity is needed to isolate the mechanisms underpinning the particular motor and cognitive characteristics associated with TS and to contrast these with mechanisms underpinning dopaminergic disorders involving fronto-subcortical circuits with differing cognitive profiles, such as Parkinson's disease.

Other studies examining inhibitory function in groups with mixed TS have reported intact performance on the Stroop test, both for adults (Channon et al., 1992) and for children (Ozonoff & Jensen, 1999); a near significant difference was reported in adults by Silverstein, Como, Palumbo, West, and Osborn (1995), suggesting poorer performance in the small subset with TS+ADHD. Georgiou, Bradshaw, Phillips, Bradshaw, and Chiu (1995) used a task with five conditions of increasing complexity, including choice RT and congruent and incongruent stimuli comparable with Stroop stimuli. Georgiou et al. reported impairment in an adult group with mixed TS in conditions involving response conflict. On a different measure (negative priming), impairment has been reported in groups of children with mixed TS (Ozonoff, Strayer, McMahon, & Filloux, 1998; Swerdlow, Magulac, Filion, & Zinner, 1996). Ozonoff et al. (1998) found that impairment was confined to a subgroup with comorbid ADHD or OCD, whereas Swerdlow et al. (1996) did not find differences between small comorbid subgroups or in adults with mixed TS. Baron-Cohen, Cross, Crowson, & Robertson (1994) reported impairments in children with mixed TS on two tasks involving hand alternation and yes–no inhibition. On go/no-go inhibition tasks, no differences were reported in correct responding in children with TS alone or mixed TS (Ozonoff,

Strayer, McMahon, & Filloux, 1994; Shucard, Benedict, Tekok-Kilic, & Lichter, 1997). Lower hit rates were reported by Sherman, Shepard, Joschko, and Freeman (1998), particularly in children with comorbid ADHD but not OCD symptomatology. There are thus some indications of impairment in inhibitory aspects of executive functioning in participants with TS, which in some instances appears greater in participants with comorbid symptomatology than in those with TS alone, as in the present study. Because impairment in TS alone appears to be relatively mild, whether or not it is detected in a given study may depend on factors such as the sensitivity of the particular tasks used.

In summary, the present findings reexamined the issue of impairment in TS and associated comorbid disorders using a wider range of measures than many previous studies have to look at several aspects of executive functioning and both explicit and implicit memory. This study provided further support for the hypothesis that impairment in TS alone is confined to inhibitory aspects of executive function, and this was detected on only one of the inhibitory measures. Somewhat more marked performance deficits in aspects of executive function were associated with comorbid ADHD symptomatology. This study highlights the importance of examining comorbid symptomatology carefully in TS both to aid the understanding of brain function in different disorders and to identify potential difficulties in functioning in everyday educational and work environments.

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Original Article

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Real-life–type Problem Solving in Tourette Syndrome

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Abstract

Objective:
The main objective of the study was to examine social problem solving in real-life–type situations in Tourette syndrome (TS).

Background:
Studies of cognitive functioning in TS have usually focused on nonsocial, abstract tasks, with mixed findings as to whether there is evidence of impairment in executive functions in those without comorbid disorders. The current study focuses primarily on social functioning, using a problem-solving task known to be sensitive to frontal lobe lesions.

Methods:
TS participants without comorbid diagnoses were compared with matched healthy control participants on a problem-solving task, using a range of interpersonal problem scenarios presented on video. A set of more abstract executive tests was also included.

Results:
Participants with TS were found to perform below a matched control group on the problem-solving task both in generating a range of potential problem solutions, and in selecting appropriate final solutions. They also performed more poorly on aspects of executive function.

Conclusions:
This study provides evidence of difficulties in both social and nonsocial aspects of functioning in TS. The implications of the findings for our understanding of TS and problem solving are discussed.

Key Words: Executive functions, Frontal lobes, Reasoning, Social problem-solving, Tourette syndrome.

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The study of social cognition and behavior in relation to brain dysfunction is of considerable importance, especially in relation to problem solving in everyday life. While disruption to a variety of brain areas may potentially impair aspects of everyday problem solving, interest has centered in particular on the effects of frontal lobe dysfunction. Tourette syndrome (TS) is a neurodevelopmental disorder in which the core symptoms consist of motor and vocal tics. TS has been linked to a range of everyday problems suggestive of executive deficits, including coprolalia and copropraxia, social inappropriateness (1), "disinhibited behaviors" (2), and getting into trouble with the law (1). It is commonly postulated that dopaminergic basal ganglia circuitry is implicated in TS, and that frontostriatal pathways supporting both cognitive and motor functioning are compromised (3–5), although others have suggested alternative models (6). Support for the theory of basal ganglia dysfunction comes in large part from the relative efficacy of dopamine antagonists in treating TS symptoms (5); eye movement studies, imaging, and biochemical data have provided additional, although sometimes conflicting, evidence. The current study was designed to examine the performance of participants with TS on an everyday social problem-solving task known to be sensitive to frontal lobe lesions (7). This problem-solving task was found to perform better than a battery of standardized executive tests in discriminating between those with anterior and posterior lesions.

Most cognitive studies of TS to date have focused on nonsocial, abstract tasks. Early cognitive studies (8) suggested TS to be associated with impairment in aspects of executive function, although these studies did not always screen for comorbid symptoms

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including attention deficit hyperactivity disorder (ADHD) or obsessive-compulsive disorder (OCD) which may also contribute to any impairment. Studies comparing TS participants with and without comorbid ADHD/OCD symptoms have tended to suggest more extensive impairment in those with comorbid disorder, particularly ADHD (9–12), although these findings are not always consistent (13). Pennington and Ozonoff (14) postulated that TS might be associated primarily with inhibitory dysfunction, since it is characterized by difficulties in suppressing unwanted motor movements and vocalizations. However, the evidence for inhibitory deficits in uncomplicated TS is inconsistent, using tasks such as the Stroop test (15–17). The current study used the Hayling test (18) as a measure of inhibition and strategy generation. There is little to suggest impairment in TS participants on tasks involving planning, rule-finding, and set-shifting, using tasks such as the WCST (19,20). Impairment in strategic aspects of memory has been reported in TS alone (21). The WCST, the Six Elements multitasking measure, and a story recall task were included in the current study to examine these issues.

The current study examined performance on a problem-solving task designed to be more directly linked to real-life activity. Multiple processes involving both cognitive abilities and life experience contribute to real-life performance, and traditional tests of executive function using abstract materials might therefore be expected to share processing demands with everyday problem solving. However, evidence from patients with frontal lobe lesions has emphasized that traditional executive tests are not always able to detect difficulties in everyday life (22). Tasks that capture characteristics of real-life performance appear to be more successful than traditional executive tests in detecting performance difficulties in people with frontal lobe lesions (7,23). Such characteristics include a lack of ordered, structured cues for problem solving, and the use of open-ended problems without clearcut right or wrong answers, where contextual factors need to be taken into account to make appropriate decisions. Everyday problem solving also relies more heavily than laboratory tasks on the effective use of previously acquired social and nonsocial, practical knowledge. Channon and Crawford (7) used real-life-type videotaped problem scenarios to examine frontal lobe contributions to problem solving. The task examined ability to recount the problem scenarios accurately, to generate a range of potential solutions, the quality of the potential solutions, and the quality of the final solutions selected to solve the problems. They found that people with anterior lesions, particularly left-sided ones, showed impairment in multiple aspects of performance, including those concerned with both generating potential solutions and judging solution quality. Those with posterior lesions showed more circumscribed difficulties relative to those with anterior lesions when compared with a matched healthy control group. A later study (24) used a shortened version of the task to study young people with Asperger syndrome, compared with a matched healthy control group. The group with Asperger syndrome was found to show impairment in aspects of problem-solving including both generating and selecting high-quality solutions. Their solutions differed most from those of the typically developing group in social appropriateness.

This task was used in the current study to examine problem-solving performance in TS, compared with a matched healthy control group. The problems are based on situations that most people might encounter either directly or indirectly, and are presented on video in real time. Investigation of whether problem-solving is affected in patients with TS has implications for our understanding and management to achieve optimal functioning in everyday life. The study also examined performance on a set of tasks concerned with different aspects of executive function, to see whether any impairments were found

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in a TS group who did not have comorbid disorders.

METHODS

Participants and Procedures

Twenty-one participants (18 men, 3 women) who met DSM-IV^{TR} criteria (25) for TS and had no other disorder took part in the study. To establish the diagnosis and rule out any comorbid disorders such as ADHD and OCD, participants were assessed against DSM-IV^{TR} criteria by a clinician with extensive experience in the diagnosis of TS and related disorders (MMR). An updated version of the National Hospital Interview Schedule for Gilles de la Tourette syndrome (26) was used to evaluate participants' TS and comorbid symptoms, using DSM-IV^{TR} criteria. This instrument was designed specifically for TS research, and is widely used in the UK. The original study showed that it had good concurrent validity when compared with another widely used instrument, the Yale Schedule (27). It provides comprehensive information on the presence and severity of characteristic symptoms of TS, and about possible comorbid behaviors including obsessive-compulsive and ADHD symptoms, and also psychosis. Participants were also interviewed regarding their educational and medical history. Those who met DSM-IV^{TR} criteria for ADHD, OCD, or any other comorbid psychiatric disorder were excluded from the study, as were those with any history of learning disability, or physical illness or injury that might have affected brain function. Inclusion criteria were fluency in English, age between 18 and 65 years of age, and a Verbal IQ score of 80 or above on the NART (28).

Twenty-one healthy control participants (17 men, 4 women) also took part in the study. The TS and control groups did not differ significantly (p = 0.280) in age (TS mean 32.90, SD 10.66; control mean 36.71, SD 11.86), years of education (p = 0.407) (TS mean 13.00, SD 2.30; control mean 13.57, SD 2.11); or NART IQ (p = 0.536) (TS mean 106.19, SD 11.50; control mean 108.29, SD 10.19). All participants gave written informed consent for the study, and were given breaks between tasks as necessary, to avoid fatigue.

Predicaments Task

The task described by Channon and Crawford (7) consisted of sixteen brief scenarios involving everyday awkward situations (see Appendix 1 for an example). Each was intended to assess the individual's ability to generate possible solutions. They also examined ability to select solutions that show appreciation of the pertinent aspects of the predicaments, and to solve them in a socially appropriate and effective manner. The situations include a range of social relationships, such as family, work colleagues, and strangers, and the scenarios take place in a range of work and social settings. A fuller description of the methodology is given in Channon and Crawford (7). Since the task was relatively lengthy to administer, Channon et al. (24) developed a shortened version which they used to study young people with Asperger syndrome and a matched control group, selecting for inclusion the eight items that best discriminated between frontal and control participants in the previous study. The shortened version was used in the current study, and has also been used in a study comparing healthy older and younger adults (29). Channon and Crawford (7) compared videotaped and story versions of the scenarios, and found that performance did not differ according to which mode of presentation was used. Videotaped presentation was therefore used for all participants in the current study. Participants were asked to watch each situation, and then to answer a series of questions.

Number of Prompts for the Problem Situations

After each problem situation was presented, participants were given an opportunity to see it again. Memory for the characters

and overt actions was assessed by asking them to describe the scenario. Unless they recalled all the main facts, they were prompted again and shown the video again if necessary. If the answer remained unsatisfactory after this, a verbal summary of any missing details was provided, giving up to three prompts in all to ensure awareness of the factual details of the awkward situation.

Awkwardness Ratings

For each problem situation, participants were asked to rate the degree of awkwardness of the situation, both for the main character, and for themselves if they were in that situation (0–100%).

Solution Generation

For each problem situation, participants were asked to generate as many potential solutions as possible within a 2-minute period. They were not given any prompts for further information or clarification of their suggested solutions, to ensure that no cues were provided as to the adequacy of their responses. To assess how efficiently people generated solutions, the number of ideas generated for each problem situation was added (number of solutions). Each of these ideas was scored for quality as described below, and scores were divided by number of solutions to adjust for how many were generated, giving generation problem appreciation, generation social appropriateness, and generation practical effectiveness scores.

Selection of Optimal and Personal Solutions

After generating possible solutions, participants were asked to select the best solution from the perspective of the main character (optimal solution). They were then asked to state what they themselves would do if they were in that situation (personal solution), to examine to what extent they chose a different course of action from that which they considered to be optimal for the main character.

Scoring of Quality for Solution Generation and Optimal and Personal Solutions

Responses were scored according to three criteria (see below) as to whether they were judged to show adequate appreciation of the problem, to be socially appropriate, and to provide effective practical means of resolving the problem. An example of a problem is shown in Appendix 1.

1. **Problem appreciation:** This measure assessed whether the solution demonstrated adequate recognition of the pertinent interpersonal/practical aspects of the problem situation that needed to be taken into account for a satisfactory outcome to be possible (scored 1 or 0).
2. **Social appropriateness:** This measure was used to classify solutions according to whether the manner of dealing with the situation was socially appropriate (scored 1 or 0).
3. **Practical effectiveness:** This was used to classify solutions according to whether the manner of dealing with the situation was likely to provide an effective practical means of resolving it (scored 1 or 0).

Ratings

All responses for the study were rated by one rater who was blind to the identity and group membership of the participants, and by a second rater who was not blind. The two raters agreed for 90.2% of ratings. All differences were resolved by reference to an additional blind rater. This was similar to the inter-rater reliability agreement reported in previous studies using this task, which ranged between 87% and 94%.

Satisfaction Ratings

After giving their Optimal and Personal Solutions, participants were asked to rate their degree of satisfaction with each (0–100%).

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Judgment of Alternative Solutions

After participants had completed each set of eight situations, they were assessed on their judgment of alternative solutions. Five alternative solutions were presented for each problem situation, and participants were asked to order them from the best to the worst solutions. Their rank orderings were scored by comparing them with the optimal rank ordering derived from Solution Quality scores (7). For each problem situation, the rank position of each answer scored between 0 and 4, according to its distance from the optimal rank position; this gave a maximum score of 20, where all 5 answers were in the correct position relative to the other 4. The maximum Judgment of Alternatives score was thus 160.

Executive Tests

A battery of executive tests was selected to assess aspects of executive function including:

1. **Inhibition and Strategy Generation:** The Hayling test (18) involved completing sentences as quickly as possible with words that make sense (part A) and with nonsensical words (part B). Patients with frontal lobe lesions have been found to have difficulty inhibiting straightforward completion words to generate nonsensical completion words in part B of the task.
2. **Multitasking:** The Six Elements test (23) was used to assess ability to organize and plan appropriate responses. In this test, participants are asked to attempt each of a set of six tasks within a 10-minute period, with certain rule constraints regarding the order in which the tasks are performed. Patients with frontal lobe lesions have been found to have difficulties in electing to alter their behavior appropriately in the absence of environmental cues to maximize gains.
3. **Rule-finding/Set-shifting:** The Wisconsin Card Sorting Test (30) involved matching response cards to four key cards on the basis of shape, color, or number, using feedback to infer the rule currently in operation. The test has been found to be particularly sensitive to dorsolateral prefrontal lesions, although other lesions may also produce impairments (31).
4. **Strategic Encoding/Retrieval:** The AMIPB Story Recall test (32) involved listening to a story, repeating it immediately, and recalling it after a filled time delay. Although posterior regions are critically involved in memory, the frontal lobes are known to play a key role in strategic encoding and retrieval, particularly for recall memory (33).
5. **Dysexecutive Behavior Ratings:** The Dysexecutive questionnaire (DEX; 34) involved self-ratings and other-ratings from a relative/friend giving information about everyday life dysexecutive problems including emotion and personality, motivation, behavior and cognition.

RESULTS

A 5% significance level was adopted throughout to compare the two groups. Data that did not meet assumptions of normality were transformed as appropriate prior to analysis, and nonparametric tests were used as necessary. There were occasional missing data points on some tests for individuals as a result of administrative errors; numbers for each group are shown in the Tables if these fall below 21.

Predicaments Task

Mean scores, standard deviations, and results of significance tests for scores on the Predicaments task are shown in Table 1. ANOVA, *t* tests, or Mann–Whitney U tests were carried out as appropriate for each of the measures.

Number of Prompts for the Problem Situations

The number of prompts needed to recount the factual details of the situations was

TABLE 1. Mean scores and standard deviations for the predicaments test

	TS group (n = 21)	Control group (n = 21)	
	Mean (SD)	Mean (SD)	Significance
Number of Prompts/3 per item	0.57 (0.48)	0.47 (0.34)	NS
Awkwardness Ratings/100%*		Group × task NS, group $p < 0.05$	
Main character	69.13 (12.77)	62.16 (13.87)	
Self	59.91 (15.18)	47.57 (17.07)	
Solution Generation			
Total number of solutions	27.05 (8.72)	33.71 (10.92)	$p < 0.05$
Generation problem appreciation/1	0.79 (0.07)	0.83 (0.05)	$p < 0.05$
Generation social appropriateness/1	0.54 (0.11)	0.57 (0.10)	NS
Generation practical effectiveness/1	0.52 (0.11)	0.54 (0.09)	NS
Optimal/Personal problem appriciation		Group × task NS, group $p < 0.01$	
Optimal/8	7.05 (0.92)	7.71 (0.64)	
Personal/8	6.86 (0.96)	7.67 (0.66)	
Optimal/Personal social appropriatness		Group × task NS, group $p < 0.05$	
Optimal/8	5.62 (1.12)	6.52 (0.93)	
Personal/8	5.33 (1.43)	6.14 (1.24)	
Optimal/Personal practical effectiveness		Group × task NS, group $p < 0.05$	
Optimal/8	5.95 (1.16)	6.57 (1.69)	
Personal/8	5.62 (1.56)	6.71 (1.35)	
Satisfcation ratings/100%*		Group × task NS, group NS	
Optimal	82.40 (9.76)	77.20 (14.59)	
Personal	84.93 (10.92)	80.16 (12.69)	
Judgement of alternatives†	125.67 (8.27)	127.62 (8.73)	NS

*N = 21 TS, 20 Control; †N = 19 TS, 21 Control.
NS, not significant.

low for both groups, and did not differ significantly ($z = 0.38$, $p = 0.704$).

Awkwardness Ratings

ANOVA was used to compare the groups in their ratings of the awkwardness of the situations from the perspective of the main character and for themselves. The group by perspective interaction was not significant ($F = 2.24$, $df = 1,39$, $p = 0.143$). There was a significant effect of group ($F = 5.14$, $df = 1,39$, $p = 0.029$), and the TS group rated the situations to be significantly more awkward than did the control group.

Solution Generation

Number of Solutions was assessed by counting the total number of solutions generated across all problem situations. t tests revealed that the TS group produced significantly fewer solutions than the control group ($t = 2.19$, $df = 40$, $p = 0.035$). The quality of the solutions generated was also examined by rating each solution for Problem Appreciation, Social Appropriateness, and Practical Effectiveness as described above, and dividing by Number of Solutions, to derive an average score. The groups differed in quality for Generation Problem Appreciation ($t = 2.52$, $df = 40$, $p = 0.016$), but not for Generation Social Appropriateness ($t = 1.12$, $df = 40$, $p = 0.268$) or Generation Practical Effectiveness ($t = 0.83$, $df = 40$, $p = 0.411$). Pearson correlations were carried out to examine the relationships between the average solution quality measures and solution generation. These showed significant correlations with Number of Solutions for Generation Practical

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Effectiveness ($r = -0.46$, $p = 0.035$) for the TS group, and with Generation Social Appropriateness ($r = -0.65$, $p = 0.001$) and Generation Practical Effectiveness ($r = -0.63$, $p = 0.002$) for the control group, showing that smaller numbers of solutions generated tended to be higher in quality.

Selection of Optimal and Personal Solutions

For each problem situation, participants were asked to select an optimal solution from the perspective of the main character, and then to give their own personal solution. These were rated on Problem Appreciation, Social Appropriateness, and Practical Effectiveness. For Optimal and Personal Problem Appreciation scores, ANOVA showed no significant group by perspective effect ($F = 0.53$, $df = 1,40$, $p = 0.471$). There was a significant effect of group ($F = 10.31$, $df = 1,40$, $p = 0.003$), showing the TS group to perform more poorly than the control group. A similar pattern was seen for Optimal and Personal Social Appropriateness scores. ANOVA showed no significant group by perspective effect ($F = 0.10$, $df = 1,40$, $p = 0.751$). There was a significant effect of group ($F = 6.51$, $df = 1,40$, $p = 0.015$), again showing the TS group to perform more poorly than the control group. Similarly, ANOVA showed no significant group by perspective effect ($F = 1.30$, $df = 1,40$, $p = 0.261$) for Optimal and Personal Practical Effectiveness scores. There was a significant effect of group ($F = 4.65$, $df = 1,40$, $p = 0.037$), with the TS group again scoring below the control group.

Satisfaction Ratings

Although the TS group scored below the control group on each of the measures of Optimal and Personal solution quality, the groups did not differ in the levels of satisfaction expressed with their Optimal and Personal Solutions. ANOVA showed no significant group by perspective effect ($F = 0.13$, $df = 1,39$, $p = 0.719$); nor was there a significant effect of group ($F = 1.97$, $df = 1,39$, $p = 0.169$).

Judgment of Alternative Solutions

To examine whether participants showed any difficulties in judging the adequacy of solutions even when they were not required to generate them, participants' rankings of five alternative solutions for each problem situation were examined. Comparison of the groups showed no significant differences in Judgement of Alternatives scores ($t = 0.60$, $df = 38$, $p = 0.550$).

Executive Tests

Mean scores, standard deviations and significance tests for the groups are shown in Table 2 *t* tests or Mann-Whitney U tests were carried out as appropriate for each of the neuropsychological tests, or ANOVA for tests involving two parts (Hayling and Story Recall).

Inhibition and strategy generation

On the Hayling test, part B error scores were higher for the TS group ($z = 1.96$, $p = 0.050$). When time taken for part A and part B was examined, ANOVA showed that the effect of group was not significant ($F = 2.83$, $df = 1,40$, $p = 0.100$), but the group by task effect approached significance ($F = 3.48$, $df = 1,40$, $p = 0.069$). Mean scores showed that the TS group was similar to the control group on straightforward sentence completion (part A), and tended to be slower on nonsensical sentence completion, involving response inhibition and strategy generation (part B).

Multitasking

The control group was at ceiling level on the test, and thus significance testing was not performed. The TS group approached ceiling level on the test.

Rule-finding/Set-shifting

The groups did not differ in the number of correct categories achieved on the WCST

TABLE 2. Mean scores and standard deviations for the executive tests

	TS (n = 21)	Control (n = 21)	
	Mean (SD)	Mean (SD)	Significance
Inhibition and Strategy Generation			
Hayling Test—Time		Group × task NS, group NS	
Time A	19.90 (4.43)	19.57 (5.00)	
Time B	37.71 (16.85)	33.24 (40.63)	
B Error score	6.86 (6.09)	3.62 (3.11)	$p < 0.05$
Multitasking			
Six Elements test			
N of tasks attempted	5.71 (0.72)	6.00 (0.00)	
Rule-finding/Set-shifting			
Wisconsin Card Sorting test			
N of categories	5.43 (1.33)	5.57 (1.16)	NS
Perseverative errors	6.57 (7.94)	5.29 (6.54)	NS
Strategic Encoding/Retrieval			
Story recall		Group × task NS, group $p < 0.05$	
Immediate	37.57 (9.91)	42.71 (6.81)	
Delayed	32.57 (12.22)	40.52 (7.19)	
DEX			
Self-rating	23.81 (9.10)	20.62 (8.34)	NS
Other-rating*	29.27 (14.85)	19.20 (9.65)	$p < 0.05$

*N = 15 TS, 15 Control.
NS, not significant.

(z = 0.07, $p = 0.941$), nor in the number of perseverative errors (z = 0.81, $p = 0.416$).

Strategic Encoding/Retrieval

There was a significant effect of group (F = 6.11, $df = 1,40$, $p = 0.018$) on Story Recall; the effect of group X time of testing was not significant (F = 1.63, $df = 1,40$, $p = 0.209$), suggesting that the TS group recalled less material than the control group both immediately after presentation and after a time delay.

DEX

For the DEX questionnaire, all participants filled out self-ratings, and scores did not differ significantly between the groups (t = 1.18, $df = 40$, $p = 0.243$). Other-ratings were returned for 15 participants in each group, and these showed a significant group difference, such that the TS participants were judged to show more dysexecutive behaviors than the control group (t = 2.20, $df = 28$, $p = 0.036$).

Correlations Between Executive Measures and Predicaments Task

Spearman rank correlations were examined for each group separately between the executive measures that differentiated the groups (Hayling error score, Story Recall and DEX-other) and the problem-solving measures that differentiated the groups (Solution Generation and Final Solution Quality scores). No significant correlations were found between the measures for either group.

Effects of Medication

Thirteen of the TS group were taking medication at the time of testing. Eleven of them were taking antipsychotic preparations, two of these in combination with a tricyclic, one with an SSRI, one with an SSRI and benzodiazepine, and one with an anticholinergic. One TS participant was taking an SSRI alone, and one an antisympathetic drug alone. The effects of medication on performance were examined in relation to the measures that had significantly differentiated the

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TS group from the control group (combining across Personal and Optimal Solutions, since there were no significant effects of perspective), although the findings must be treated with caution since power was low. Comparison of the medicated TS subgroup (N = 13) with the control group showed them to be significantly poorer on Number of Solutions (p = 0.033), Optimal/Personal Problem Appreciation (p = 0.002), and Optimal/Personal Social Appropriateness (p = 0.045), Hayling errors (p = 0.014) and Story Recall (p = 0.034). Comparison of the unmedicated TS subgroup (N = 8) with the control group showed them to be significantly poorer on Generation Problem Appreciation (p = 0.022), Optimal/Personal Problem Appreciation (p = 0.049) and Optimal/Personal Social Appropriateness (p = 0.036), and DEX-other ratings (p = 0.048). Direct comparison of the medicated and unmedicated TS participants did not reveal any significant effects on any of the measures ($p > 0.05$).

DISCUSSION

The main findings were that on the Predicaments task, the TS group was significantly poorer on several aspects of performance, generating fewer potential problem solutions and selecting poorer final solutions. They also judged the scenarios to be more awkward than the control group, and did not differ in their satisfaction with their own performance. With respect to the executive tests, the TS group differed significantly from the control group in the number of Hayling errors and on Story Recall. The TS participants were also rated by others as displaying more dysexecutive behaviors on the DEX questionnaire. There was no evidence to suggest that the findings were attributable to the effects of medication.

When solution quality was examined for the final optimal and personal solutions selected, the participants with TS scored significantly below the control group in each aspect of solution quality rated, both for optimal and personal solutions. These included ability to appreciate the pertinent interpersonal/pracical aspects of the situations, and to devise solutions judged likely to be socially appropriate and effective. On the problem appreciation measure, the control group but not the TS group approached ceiling levels. This is potentially a critical aspect of problem-solving performance, since it assesses ability to take into consideration the social and practical nuances of the problem situation in selecting an appropriate solution. Weaker performance in the TS group on this measure may reflect a failure to make adequate use of prior knowledge, affecting ability to identify appropriate goals and look ahead to the potential consequences of different courses of action. Solution quality also differentiated the groups for the problem appreciation measure when fluency solutions were examined. Participants with anterior lesions studied by Channon and Crawford (7) were also characterized by impaired solution quality, both for solutions they generated themselves and when asked to judge solutions that they did not generate. By contrast, those with Asperger syndrome (24) had difficulties predominantly in relation to a single aspect of solution quality, namely producing solutions that were socially appropriate. For the current study, there was no evidence of difficulties in judging solution quality when participants with TS were asked to judge given solutions rather than generate them. However, this is probably a less sensitive measure than the selection of optimal and personal solutions, since the alternatives presented for each problem reflected relatively large differences in quality, in that some of the five solutions for each problem could easily be judged to be very poor. The presentation of alternatives which all provided at least partially acceptable solutions would have offered a more subtle test of comparative judgment of abstract solutions not generated by the participants themselves. The current findings may therefore underestimate the extent of difficulties in making such judgments in the TS group.

The TS group did show clear evidence of impairment on the more stringent measures of problem-solving performance reflected in the quality of their self-generated final solutions. Despite this, it is of interest that they did not rate satisfaction with their final solutions any lower than did the control group. Failure to perceive or to report differences in satisfaction may reflect a subtle degree of impairment in awareness or acknowledgment of their performance, which may in turn be associated with similar failure to appreciate difficulties in everyday life. To our knowledge, problem-solving performance has not previously been studied in people with TS, although there are some indications of difficulties associated with behavior in everyday social situations. For instance, some individuals with TS show socially inappropriate behavior, in addition to the long-recognized symptoms of coprolalia and copropraxia associated with the disorder (1). There is also work suggesting that children with TS are at higher risk for having poor peer relations than a clinical control group (35,36). The DEX questionnaire was included to give an indication of everyday life behavior, and this also provided some support for the view that insight might be poorer in the TS group. Although the groups did not differ significantly in their self-ratings of behavior in everyday life on this instrument, other-ratings judged the TS participants to display significantly more dysexecutive behaviors than the control participants.

The participants with TS showed impairment on the Predicaments test not only in the quality of the solutions they produced, but also in the number of possible solutions that they generated. This is a potential source of difficulty in relation to final solution quality, since failure to generate adequate solutions could clearly contribute to poor selection of optimal and personal solutions. However, ability to generate a range of possible solutions did not correlate significantly with the final solution quality measures for either group. Presumably individuals used different strategies in generating potential solutions, with some individuals producing a small number of possible solutions that were nevertheless high in quality. There was a relationship between solution fluency and the average quality of the solutions generated for the control participants on two of the three quality measures, and for the TS participants on one of the quality measures. This showed that those who generated more solutions tended to score lower in the average quality of those solutions, perhaps because they had exhausted the possible range of good solutions to the problems in generating higher numbers of ideas.

Real-life problem solving in everyday situations involves appropriate selection of information as a focus of attention, identifying appropriate goals, looking ahead to potential future consequences of different courses of action, making reasoned comparative judgments about them, and evaluating performance. A set of abstract executive tests was also included in the current study, since nonsocial executive processes may share common ground with real-life social problem solving. However, scores on these executive tasks were not correlated with scores on the Predicaments measures that differentiated the groups. This may indicate that differences in problem solving between the groups were primarily related not to these types of executive processes, but to other factors such as emotional processes or knowledge structures. The TS participants rated the problem situations as significantly more awkward than did the control participants, which may have indicated a stronger emotional response to the problems in the TS group. It is possible that emotional arousal interfered with their ability to generate adequate solutions, or that impairment in other aspects of emotional processing contributed to their difficulties in problem solving. Recent brain-imaging methodology has linked lateral frontal lobe areas to executive processes such as working memory and inhibition (37), whereas orbitomedial frontal lobe areas are thought to be associated with emotional learning processes based on reward

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and punishment. Bechara et al. (37–39) postulate that somatic/emotional markers may play a crucial role in supporting cognitive decision-making processes, and that these are compromised in patients with lesions to ventromedial frontal cortex. These markers are said to provide cues, overtly or covertly, signalling previous learning of reward or punishment based on prior experience, and thereby influence performance before conscious knowledge is available to guide reasoning. Rolls (40) also argues that areas of frontal cortex, particularly orbital regions, are involved in modulating behavior according to experience of reward and punishment, based on tasks such as reversal learning. Specific difficulties in making appropriate use of emotional information were thought to underpin "acquired sociopathy" in a single case study by Blair and Cipolotti (41) concerning a man with a right frontal lesion including the orbitofrontal cortex. Somatic/emotional components in addition to cognitive components represent another source of potential impairment for TS participants in the current study, although no direct evidence was available to evaluate this.

The TS group performed at similar levels to the control group on some of the executive measures, but did differ on measures of inhibition/strategy generation and strategic memory. The Hayling test is thought to be a sensitive measure of response inhibition and strategy generation, known to be impaired after frontal lobe lesions (18). This is of interest in view of debate as to whether uncomplicated TS is associated with impaired executive functioning (14). The TS group was also poorer on story recall, a task that involves both executive processes of strategic encoding and retrieval, and long-term memory storage. Further work is needed to explore the particular difficulties underlying memory performance. Overall, these findings provide some support for the notion that uncomplicated TS is associated with deficits in inhibitory aspects of executive function. However, although none of the participants in the current study met criteria for comorbid disorders, it is possible that subclinical comorbid symptoms contributed to performance, in addition to TS symptoms. This could be explored further in a future study using self-report/observer ratings to examine any correlations between task performance and TS/OCD/ADHD symptoms.

To what extent can it be inferred that performance on the Predicaments test predicts real-life problem-solving behavior? Experimental tests typically provide structured cues for performance that are lacking in real life, leading to underestimation of everyday difficulties. The use of open-ended problem situations and limited use of questions and prompts in the current task was designed to minimize this. Conversely, everyday problems might also be overestimated by measures of this type as a result of difficulty imagining the situations in reality, or limited personal investment in the outcome. Nevertheless, the ecological validity of the task was thought to be high because successful solution required participants to take into account multiple contextual factors in anticipating potential outcomes, and there were no clearcut right or wrong answers. Moreover, the problems were based on real-life situations. The findings from the DEX questionnaire provide some evidence for the validity of the test, since other-ratings indicated that the TS group showed significantly more dysexecutive features in everyday life than the control group.

In summary, the current findings show that real-life–type problem-solving performance was impaired for participants with TS, who were poorer both in generating a range of potential solutions, and selecting socially appropriate and practically effective final solutions. They also performed below the level of the control group on more abstract executive tasks, but a lack of correlations between the executive and social problem-solving measures raises the possibility that they may have dissociable cognitive and neural bases relating to separate frontosubcortical pathways. The development of clinical tools based on measures such as the current prob-

lem-solving task may aid in the clinical assessment of individuals with TS, helping to identify any difficulties in complex situations in real life.

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APPENDIX 1

An Example of a Predicament: "Dogs"

Anne is in her office when Tony comes in. She asks how he is, and he says he is alright, but tired. She agrees that he looks tired, and asks what is the matter. He has new neighbors who moved into the flat above his a couple of weeks ago. They are nice people, but they own dogs and keep them in their kitchen at night, which is directly above Tony's bedroom. All night, and every night since they moved in, the dogs jump around and bark. He finds it impossible to get to sleep. He says he has had a word with the neighbors, and although they were very reasonable, they said they had nowhere else to put the dogs as it is a block of flats.

Examples of Good Solutions

Discuss it again with the neighbors and negotiate a compromise.

Take it up with the council/landlord.

Scoring of Solution Quality

1. Problem Appreciation
   The scoring categories were classified as follows:
   (A) Attempt to negotiate a solution with the neighbors.
   (B) Make further complaints (e.g. go to landlord/council/police).
   (C) Alter your own life (e.g. earplugs, move house).
   (D) Extreme ideas (e.g. kill the dogs).
   (E) Irrelevant or incomplete responses (e.g. people shouldn't keep dogs in flats).

   Categories A, B, and C in this example were assigned a score of 1 to indicate adequate appreciation of the pertinent issues; categories D and E were assigned a score of 0, to indicate poor appreciation of the issues. These scores were assigned whether solutions were socially appropriate or effective.
2. Social Appropriateness
   The scoring categories were classified as follows: Category A answers scored 1 point, category C, D, and E answers scored 0 points, and category B answers were split according to the degree of social appropriateness indicated (e.g. aggressive or threatening answers scored 0).
3. Practical Effectiveness
   The scoring categories were classified as follows: Category D and E answers scored 0 points, and category A, B, and C answers were split into those scoring 1 or 0 according to the degree of practical effectiveness indicated.

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Social Cognition in Tourette's Syndrome: Intact Theory of Mind and Impaired Inhibitory Functioning

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Although associations between social cognition involving theory of mind and non-social executive skills have frequently been reported, dissociations in performance have also been found. The present study was designed to examine social and non-social cognition in uncomplicated Tourette Syndrome (TS). Adult TS participants without comorbid diagnoses were compared to matched healthy control participants on social cognition measures involving theory of mind and empathy, and on non-social executive tasks. Participants with TS were found to make more errors than a matched control group on an inhibitory task, but did not differ on other executive measures or on the social cognition measures. The implications of the findings for our understanding of TS and of the relationship between social cognition and executive skills are discussed.

KEY WORDS: Theory of mind; mentalising; inhibition; social cognition; Tourette's syndrome.

Controversy currently surrounds the nature of the relationship between theory of mind and executive functioning, where associations in performance have often been reported. This may reflect the potential contribution of both executive and theory of mind skills to social cognition tasks, providing a dual route to impairment. Alternatively, there have been proposals linking the development of theory of mind and executive skills, suggesting variously that executive functioning may underpin theory of mind, that theory of mind may underpin executive functioning, or that embedded conditional reasoning may support both executive functioning and theory of mind (for a discussion see e.g., Perner & Lang, 1999; Hughes, 2002). Impairment on both social cognition tasks involving theory of mind and on certain executive tasks has been reported in a range of studies including young typically developing children (e.g., Carlson, Moses, & Breton, 2002; Frye, Zelazo, & Palfai, 1995), children with autism (e.g., Zelazo, Jacques, Burack, & Frye, 2002), and adults with frontal lesions (e.g., Channon & Crawford, 2000).

Neuroanatomical proximity could also account for the co-occurrence of deficits in both theory of mind and non-social executive domains, implying that each can be selectively impaired. Dissociations between performance in these two domains provide some support for this contention. For instance, single cases have been reported to show impairment on tasks involving theory of mind but preserved performance on executive tasks, including a patient with early left amygdala damage (Fine, Lumsden, & Blair, 2001) and one with frontal variant frontotemporal dementia linked to orbitofrontal cortex (Lough, Gregary, & Hadges, 2001). The converse dissociation of intact theory of mind and impairment in aspects of executive functioning has been reported in a patient with orbitofrontal damage (Bach, Happé, Fleminger, & Powell, 2000) and in children at risk of attension deficit hyperactivity disorder (ADHD) (Perner, Kain, & Barchfeld, 2002).

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The concept of empathy has commonly been used with respect to emotional processes involved in identifying with others' feelings, although it potentially encompasses both emotional and cognitive components (see e.g., Eslinger, 1998 for a review). Marked changes in empathic ability have been reported in people with frontal lesions and in those with traumatic brain injury (e.g., Grattan, Bloomer, Archambault, & Eslinger, 1994). Both lesion studies (e.g. Channon & Crawford, 2000; Rowe, Bullock, Polkey, & Morris, 2001; Stone, Baron-Cohen, & Knight, 1998; Stuss, Gallup, & Alexander, 2001) and functional imaging studies (e.g., Baron-Cohen *et al.*, 1994, 1999; Brunet *et al.*, 2000; Fletcher *et al.*, 1995; Gallagher *et al.*, 2000; Goel, Grafman, sadato, & Hallet, 1995) have suggested frontal lobe involvement in theory of mind; medial/orbital frontal regions in particular have been implicated in these studies, although other regions such as the amygdala may also be involved.

Taken together, the findings suggest that social cognition is potentially independent of non-social executive abilities, at least in the mature brain. In the present study, this contention was explored further by investigating social cognition and executive functioning in adults with Tourette's syndrome (TS). TS is a neurodevelopmental disorder involving motor and vocal tics. Fronto-striatal pathways are believed to be compromised (e.g. Leckman *et al.*, 1997; Moriarty *et al.*, 1997), and there has been considerable recent interest in the question of whether there are associated impairments in cognition as well as in motor functioning. Early studies suggested TS to be associated with impairment in aspects of executive function, although these studies did not always screen for comorbid symptomatology including ADHD or obsessive-compulsive disorder (OCD) which may contribute to any impairment (see e.g., Pennington & Ozonoff, 1996). Current evidence based on studies that have screened for comorbidity points to a selective executive deficit in inhibitory skills. Impairment on inhibitory tasks has been reported in those with TS-alone (e.g., Channon, Crawford, Vakili, & Robertson, 2003a; Channon, Pratt, & Robertson, 2003b; Sherman, Shepard, Joschko, & Freeman, 1998), although not invariably. There is little evidence of impairment in TS-alone in skills including planning, rule-finding or set-shifting (e.g., Pennington & Ozonoff, 1996); these skills have been linked to regions including dorsolateral prefrontal cortex (PFC) (e.g., Cabeza & Nyberg, 2000).

Relatively little attention has been paid to the question of social cognition in TS. From an everyday perspective, there are some indications of social difficulties associated with TS including coprolalia and copropraxia, social inappropriateness (Kurlan *et al.*, 1996), "disinhibited behaviors" (Cohen & Leckman, 1992), and getting into trouble with the law (Kurlan *et al.*, 1996), although these are not always present and may be more marked when there is comorbid symptomatology. It is currently unclear whether these arise from the same inhibitory deficit that appears to affect performance on non-social executive tasks, or whether there are deficits in aspects of social cognition that may reflect additional independent difficulties. One study to date has examined ability to identify mental states from pictures of people's eyes (Baron-Cohen, Jolliffe, Mortimore, & Robertson, 1997); performance was reported to be intact in a group of TS participants not screened for comorbidity; executive skills were not examined in this study. On a test of social problem solving known to be sensitive to frontal lobe lesions, Channon *et al.* (2003a) found that those with TS-alone showed impairment both generating a range of potential problem solutions, and in selecting appropriate final solutions. Difficulties in inhibiting inappropriate responses might be expected to impair ability both to generate and select high quality problem solutions. However, problem-solving performance in this study did not correlate significantly with inhibitory performance on the Hayling test, or with scores on other executive tasks, raising the possibility that impairment on the problem-solving task was linked not to inhibitory deficits, but to other skills such as impaired mentalising/empathic ability. Some tentative evidence for this possibility was provided by Channon and Crawford (2000), who found impairment on a story comprehension task involving mentalising skills in those with frontal lobe lesions; some of the group had also participated in an earlier problem-solving study that showed impairment associated with frontal lesions (Channon & Crawford, 1999).

The present study was designed to examine theory of mind and empathic ability in TS participants screened for comorbidity. Theory of mind was assessed using a story comprehension task (Happé, 1994) that has been extensively used in the literature to study performance in people with autism and also with focal lesions, and has also been used in imaging studies. A second story comprehension task (Channon & Crawford, 2000) using slightly more

difficult materials was also used. Empathy was assessed on a commonly used self-report questionnaire (Davis, 1983). Non-social executive tasks involving both inhibitory and other skills were also included. It was expected that the TS group would show impairments on the inhibitory executive task, but would be intact on the other executive tasks. No specific prediction was made regarding theory of mind and empathic skills, in view of the limited evidence available from previous literature.

METHOD

Participants and Procedure

Fifteen participants (11 m, 4 f) who met DSM-IV^{TR} criteria (APA, 2000) for TS and no other disorder took part in the study. In order to establish the diagnosis and rule out any comorbid disorders such as ADHD and OCD, participants were assessed against DSM-IV^{TR} criteria by a clinician with extensive experience in the diagnosis of TS and related disorders (MMR). An updated version of the National Hospital Interview Schedule for Gilles de la Tourette Syndrome (Robertson & Eapen, 1996) was used to aid diagnosis, using DSM-IV^{TR} criteria. This instrument was designed specifically for TS research, and is widely used in the UK. The original study showed that it had good concurrent validity when compared with another widely used instrument, the Yale Schedule (Pauls & Hurst, 1987). It provides comprehensive information on the characteristic symptoms of TS, and about possible comorbid behaviours including obsessive-compulsive and ADHD symptomatology, and also psychosis. Those who met DSM-IV^{TR} criteria for ADHD, OCD or any other comorbid psychiatric disorder were excluded from the study, as were those with any history of learning disability, or physical illness or injury that might have affected brain function. Inclusion criteria were fluency in English, age between 18 and 65 years of age, and a Verbal IQ score of 80 or above on the NART (Nelson, 1991).

Twenty-three matched healthy control participants (18 m, 5 f) also took part in the study. The TS and control groups did not differ significantly in age, (TS M = 33.87, SD = 12.31; control M = 33.78, SD = 8.23), years of education (TS M = 11.87, SD = 3.52; control M = 12.61, SD = 1.99); or NART IQ (TS M = 105.93, SD = 12.13; control M = 106.70, SD = 8.53). All participants gave written informed consent for the study.

Social Measures

Advanced Theory of Mind

These stories were developed to study higher-level theory of mind in autism by Happé (1994) and later adapted (Happé, Brownell, and Winner, 1999). They consisted of eight mentalistic (theory of mind) stories, and eight non-mentalistic control stories, presented in written form one at a time in fixed random order. The theory of mind stories were concerned with double bluff, misunderstanding, persuasion and white lies. After studying each story, participants were asked questions requiring inferences about the thoughts and feelings and sometimes the intentions of the central character. The control stories were also about people, and participants were asked questions requiring inferences about practical rather than mentalistic issues. Performance was scored 0–2 for correctness of answers, with 2 points for a fully correct answer, 1 point for a partially correct answer, and 0 for an incorrect answer. An example of each type of story is shown in Appendix 1.

Story Comprehension

This task was developed by Channon and Crawford (2000). It consisted of twelve mentalistic stories involving higher-level theory of mind and pragmatic language comprehension. Participants were asked to explain the main character's speech or actions, but the link between the words or actions and the reason behind them was not made explicit. The final sentence always contained information which ensured that the story could not be understood adequately using a literal interpretation, so that it was necessary to invoke a non-literal interpretation. The stories included examples of sarcasm, pretence, misunderstanding, lies and persuasion. Performance was scored 0–1 for correctness of answers. An example is shown in Appendix 1.

Interpersonal Reactivity Index (IRI; Davis, 1980)

This is a 28-item self-report measure assessing both cognitive and emotional aspects of empathy, including perspective-taking (the extent to which

people adopt the perspectives of others and to see things from their points of view), fantasy (the tendency to identify emotionally with fictitious situations such as books, films and daydreams), empathic concern (the extent to which people experience feelings of warmth, compassion, and concern for others) and personal distress (self-oriented feelings of fear and discomfort in the face of others' bad experiences). Each item was rated on a scale from 0–5, giving a total score of 0–140, where higher scores indicated higher levels of empathy.

Non-Social Executive Measures

Inhibition

The Hayling test (Burgess & Shallice, 1996) involved completing 15 orally presented sentences as quickly as possible with words that make sense (part A) and completing 15 sentences with nonsensical words (part B) (e.g., London is a big..."banana"). Straightforward completion errors consisted of words that made sense in completing the sentence (e.g., London is a big..."city"), and these received an error score of 3. Related completion errors consisted of words that were related or opposite in meaning to the sentence (e.g., London is a big..."village"), and these received an error score of 1. The maximum possible error score was therefore 45. Patients with frontal lobe lesions have been found to have difficulty inhibiting straightforward completion words to generate nonsensical completion words in part B of the task, and functional imaging has implicated the ventrolateral PFC and anterior cingulate.

Rule-Finding/Set-Shifting

The Wisconsin Card Sorting Test (WCST; Grant & Berg, 1948) involved matching response cards to four key cards on the basis of shape, colour or number, using feedback to infer the rule currently in operation. The correct rules were, in order: shape, colour, number, shape, colour and number. There were 128 trials, and scoring was based on the number of correct rules (i.e., 10 consecutive correct sorts) and the number of perseverative errors (i.e., sorts to the previously correct rule once feedback had been given that this was no longer correct). The test has been found to be particularly sensitive to dorsolateral PFC lesions, although other lesions may also produce impairments (Milner, 1963).

Multitasking

The Six Elements test (Wilson, Alderman, Burgess, Emslie, & Evans, 1996) was used to assess ability to organise and plan appropriate responses. In this test, participants are asked to attempt each of a set of six tasks within a ten-minute period, with certain rule constraints regarding the order in which the tasks are performed. The six tasks consisted of two sets (A and B) of written arithmetic questions, two sets (A and B) of pictures to be named, and two dictation tasks (A and B). The rules specified that sets A and B of any one type of task (arithmetic, naming or dictation) should not be carried out successively, and that all six tasks should be attempted during the allocated time. Scores were based on number of tasks completed, rule breaks and time taken, to give an overall score ranging from 1 to 4. Tasks that require strategic organisation in unstructured situations when environmental cues to guide behaviour are lacking have been reported to be sensitive to lesions involving medial polar (Burgess, Veitch, de Lacy Costello, & Shallice, 2000) and orbital PFC (Levine *et al.*, 1998). Lesions in these areas are linked to difficulties in electing to alter behaviour appropriately to maximise gains according to the reward contingencies.

RESULTS

Mean percentage scores, standard deviations and results of significance tests for the social and nonsocial measures are shown in Table I. A 5% significance level was adopted throughout to compare the two groups. All *t*-tests were two-tailed.

Social Measures

Advanced Theory of Mind

ANOVA with one within-group factor (type of story: mentalistic or control) and one between-group factor (TS or control group) showed no significant effect of group, $F(1.36) = 2.48$, $p = .124$, nor group by type of story interaction, $F(1.36) = 0.09$, $p = .770$.

Table I. Mean Percentage Scores and Standard Deviations for the Social and Non-Social Measures

	Group				
	TS (n = 15)		Control (n = 23)		
	M	(SD)	M	(SD)	Significance
Social measures					
Advanced theory of mind					NS
Mentalistic stories	87.08	(10.15)	91.03	(7.73)	
Non-mentalistic stories	82.92	(12.15)	87.77	(9.13)	
Story Comprehension[a]					
Story scores	83.93	(11.07)	83.70	(12.17)	NS
Interpersonal Reactivity Index[b]	55.38	(5.94)	56.82	(8.97)	NS
Non-Social Measures					
Inhibition					
Hayling errors	10.07	(5.87)	5.99	(5.90)	$p < .05$
Rule-Finding/Set-Shifting					
WCST N of categories	81.11	(33.85)	91.30	(20.02)	NS
Perseverative errors	6.51	(8.42)	3.46	(5.41)	NS
Multitasking					
Six Elements test	96.67	(8.80)	97.83	(7.20)	NS

Note: [a] N = 14 TS; [b] N = 22 Control.

Story Comprehension

A t-test showed no significant difference between the two groups in the number of correct stories, $t(35) = 0.06$, $p = .954$.

Interpersonal Reactivity Index

A t-test showed no significant difference between the two groups on the empathy scale, $t(35) = 0.54$, $p = .590$.

Non-Social Executive Measures

Inhibition

On the Hayling test, inhibitory error scores were significantly higher for the TS group, $t(36) = 2.09$, $t = .044$.

Rule-Finding/Set-Shifting

On the WCST, there was no significant difference between the groups in the number of categories correctly completed, $t(36) = 1.17$, $p = .250$, or in the number of perseverative errors, using logarithmically transformed scores, $t(36) = 1.17$, $p = .249$.

Multitasking

On the Six Elements test, the groups did not differ significantly, $t(36) = .44$, $p = .659$; both groups approached ceiling levels on the test.

Inhibition vs. Social and Non-Social Executive Measures

A direct comparison was made of the inhibitory measure, where the TS group performed significantly more poorly than the control group, and the other measures, which did not differentiate the groups. The variables were each z-transformed for ease of comparison. A composite measure was calculated for the social measures by averaging across the variables (Advanced Theory of Mind, Story Comprehension and Interpersonal Reactivity Index). ANOVA was then carried out comparing the two groups on the composite Social measure versus the Hayling inhibitory measure. This showed a significant group by task interaction, $F(1.34) = 5.55$, $p = .024$. This pattern remained similar when the analysis was repeated using the matching variables (age, education and IQ) as covariates, i.e., there was a significant group by task interaction ($p = .038$). A composite Non-Social measure was also calculated by averaging across the executive tests except for the

Hayling (WCST, Six Elements test). ANOVA was then carried out comparing the two groups on the composite Non-Social measure versus the Hayling inhibitory measure. This showed a significant group by task interaction, $F(1.36) = 4.67$, $p = .037$. This pattern remained similar when the analysis was repeated using the matching variables (age, education and IQ) as covariates, i.e., there was a significant group by task interaction ($p = .048$).

DISCUSSION

The results showed that as expected, the TS group was significantly poorer than the control group on an inhibitory measure, the Hayling test, compared with the other social or non-social executive measures.

The findings for the non-social executive measures are consistent with previous literature suggesting circumscribed inhibitory impairments in uncomplicated TS. The Hayling test is thought to be a sensitive measure of response inhibition and strategy generation, and performance has previously been shown to be impaired in TS (Channon *et al.*, 2003a, 2003b) as well as in frontal lobe lesions (Burgess & Shallice, 1996). Recent work examining the fractionation of functions mediated by the frontal lobes has linked performance on inhibitory tasks of this nature to regions including ventrolateral PFC and the anterior cingulate (e.g. Nathaniel-James, Fletcher, & Frith, 1997). The Hayling task required the inhibition of a prepotent oral response in favour of a non-conformist one, and appears to share qualities with the everyday life coprolalic difficulties that occur in TS, where social convention requires us to inhibit inappropriate comments in favour of more socially acceptable ones. A more detailed exploration of the specific nature of any inhibitory impairments in TS would be of interest in the future, to examine precisely what task characteristics are critical in determining the presence or absence of difficulties. The WCST was chosen in the present study primarily as a measure of rule finding and shifting, but might also have some sensitivity to inhibitory difficulties in failing to suppress a learned response set, as reflected particularly by perseverative errors. Inspection of the scores showed that the number of perseverative errors ranged from 0 to 27 in the control group, and from 0 to 32 in the TS group, but the groups did not differ significantly on this or on the number of correct rules identified. The lack of impairment on the WCST in TS participants in the present study is consistent with previous findings, which have for the most part failed to detect any impairment in TS on tasks involving planning, rule-finding or set-shifting (see e.g., Pennington & Ozonoff, 1996). The TS participants in the present study were also found to be unimpaired in multitasking.

The TS group performed at similar levels to the control group on the two tasks involving theory of mind, and on the self-report empathy questionnaire. Both the story comprehension tasks required participants to read brief interpersonal scenarios and explain the actions or words of the characters, and were therefore believed to test ability to take the perspective of another. Pragmatic language comprehension skills also potentially contributed to performance on the story tasks, i.e., ability to interpret the meaning of the words in the particular social context. Examples of relatively complex pragmatic language such as sarcasm were included, and there was no evidence that the TS group found the materials any more taxing than did the control group. With respect to the empathy questionnaire, the groups did not differ on subscales concerned with either cognitive or emotional aspects of empathy.

This suggests social cognition to be intact in uncomplicated TS, at least on skills involved in empathy and theory of mind, and this is consistent with previous work with a TS sample that reported no differences in ability to identify mental states from pictures of people's eyes (Baron-Cohen *et al.*, 1997). Channon *et al.* (2003a) did report impairment on a social problem solving task in TS, which did not correlate with performance on non-social executive tasks, but this does not in itself demonstrate that problem-solving impairment reflected deficits in social rather than non-social cognition; inhibitory difficulties may nevertheless have interfered with problem solving. It is of course possible that the present study failed to detect differences in social cognition for methodological reasons. The sample size was relatively small, and it could be argued that statistical power was therefore inadequate to detect a subtle significant effect. However, inspection of the mean scores gives little indication that collecting a larger sample size would have produced a significant difference. No direct measure of social functioning was used to assess whether the TS participants showed difficulties in everyday life. Failure to detect differences in mentalising/empathic ability may therefore simply reflect the fact that they were a relatively high functioning group from

a social everyday point of view. However, they were all recruited from a specialist tertiary clinic for TS that typically sees more severe cases than are found in community samples.

It is also possible that the measures chosen for the present study were not sufficiently sensitive to detect subtle differences as a result of ceiling effects. This issue is pertinent for simpler, second-order theory of mind tests, since typically developing children aged 6 normally pass these (Perner & Wimmer, 1985). However, the two theory of mind measures used in this study were chosen because they were designed to assess higher-order mentalising skills. The Advanced theory of mind task was originally designed for older children, and has been found to be sensitive to impairments both in adults with autism/Asperger syndrome and with focal brain lesions (e.g., Happé, 1994; Happé, Malhi, & Checkley, 2001). The other story comprehension task was designed as an adult-level task, and has been found to be sensitive to impairments in adults with focal brain lesions (Channon & Crawford, 2000). The Eyes test which showed intact performance in TS (Baron-Cohen *et al.*, 1997) was also designed to be of adult-level difficulty. There is thus little reason to suggest that failure to find differences between the groups is attributable to the use of inadequate measures.

One possible route for further exploration is the investigation of other tasks associated with medial rather than lateral frontal lobe functioning. The present study did not assess performance on tasks such as reversal learning (e.g., Rolls, 1999) or the gambling task used by Bechara and colleagues (e.g., Bechara, Tranel, Damasio, & Damasio, 1996; Bechara, Damasio, & Damasio, 2000). Such reward-learning processes have been linked to ventromedial frontal lobe areas, and are thought to be involved in modulating everyday decision-making. No evidence is currently available regarding these aspects of performance in TS, although multitasking, which was assessed and found to be intact in the present study, has been linked to both lateral PFC and to polar medial/orbital frontal regions (Burgess *et al.*, 2000; Levine *et al.*, 1998).

Overall, the most parsimonious account of the present findings is that uncomplicated TS is not associated with difficulties in social cognition involving theory of mind and empathic ability, and that any executive deficits are circumscribed, primarily reflecting impaired inhibitory skills. This study therefore provides further support for the view that social cognition including theory of mind skills can be dissociated from non-social executive skills. This is most readily interpreted in terms of distinct neuroanatomical underpinnings for the different domains, whereby associations between performance on theory of mind and inhibitory tasks are attributable to the proximity of neuroanatomical areas disrupted, rather than reflections of skills with a single shared underlying substrate. With respect to TS, the inhibitory task used in the present study required the inhibition of prepotent oral responses, and bears apparent similarities to the type of inhibitory failure that may be involved in saying socially inappropriate words in coprolalia. Closer examination of different postulated inhibitory processes is needed in TS using a range of task manipulations to determine the relevant mechanism(s) implicated. Further work could also explore whether inhibitory impairment underpins everyday difficulties linked to the disorder, both social and non-social, comparing both those with and without comorbid diagnoses. If this is indeed the case, social difficulties such as coprolalia, copropraxia and other difficulties might reflect impaired ability to inhibit responses, combined with intact social knowledge and appreciation of the inappropriateness of the behaviour in the social context.

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APPENDIX 1. ADVANCED THEORY OF MIND TASK (HAPPÉ, *ET AL.*, 1999)

Example of mentalistic story

A burglar who has just robbed a shop is making his getaway. As he is running home, a policeman on his beat sees him drop his glove. He doesn't know that man is a burglar, he just wants to tell him he dropped his glove. But when the policeman shouts out to the burglar, "Hey, you! Stop!", the burglar turns round, sees the policeman and gives himself up. He puts his hands up and admits that he did the break-in at the local shop.

Question: Why did the burglar do that?

Example of non-mentalistic control story

A burglar is about to break into a jewellers' shop. He skilfully picks the lock on the shop door. Carefully he crawls under the electronic detector beam. If he breaks this beam it will set off the alarm. Quietly he opens the door of the store-room and sees the gems glittering. As he reaches out, however, he steps on something soft. He hears a screech and something small and furry runs out past him, towards the shop door. Immediately the alarm sounds.

Question: Why did the alarm go off?

Story Comprehension task (Channon & Crawford, 2000)

Example

Marie dreaded her trips to meet her husband's relatives because they were so boring. Most of the time, they all sat in awkward silence, and this occasion was no different. On the way home, Marie's husband asked her how she found the visit. Marie said "Oh, marvellous. I could hardly get a word in edgeways."

Question: Why did Marie say that?

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Tourette's syndrome: performance on tests of behavioural inhibition, working memory and gambling

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Background: Tourette's syndrome (TS) is a neurodevelopmental disorder associated with fronto-striatal dysfunction. There is debate as to the extent to which TS is associated with cognitive impairment. Some authors argue that any impairments seen are attributable to comorbid psychiatric symptomatology, whilst others have suggested that uncomplicated TS is associated with mild deficits limited primarily to inhibitory processes. The present study was designed to examine this issue using carefully screened participants with TS and experimental measures thought to involve different areas of the frontal lobes. **Methods:** Adolescents with TS who were screened for comorbid psychiatric disorder were compared with a healthy control group on a set of executive measures. Two tasks involving behavioural inhibition were used: a Sentence Completion task in which sentences had to be completed first with straightforward and then with nonsensical endings, and a Flanker task in which a central stimulus was surrounded by either compatible or incompatible flankers. Working memory was assessed using an N-back task, and reward learning was assessed using a Gambling task. Both accuracy and reaction times were measured for each task. **Results:** The TS group differed significantly from the control group on both the Sentence Completion task and on the Flanker task. On the Sentence Completion task, they were slower to make both sensible and nonsensical completions, and they had higher error scores on the nonsensical completions. On the Flanker task, the TS participants were less accurate than the control group, since they were poorer on the incompatible but not the compatible trials. A similar interaction with trial type was found for speed, where the TS participants were slowed more by the incompatible versus compatible trials, although overall their performance tended to be faster than the controls. The TS group did not differ significantly from the control group on measures of working memory or reward learning. **Conclusions:** The findings provide further evidence that uncomplicated TS is not associated with widespread executive impairments. However, it was not clear that any differences between the groups could be attributed solely to selective inhibitory impairment. **Keywords:** Tourette's syndrome, frontal lobes, executive, inhibition, working memory.

Tourette's syndrome (TS) is a neurodevelopmental disorder that is characterised by the presence of a range of both motor and vocal tics. TS is also associated with a range of difficulties in everyday life such as coprolalia, copropraxia (Kurlan et al., 1996), 'disinhibited behaviours' (Cohen & Leckman, 1992) and uncontrollable urges to perform socially inappropriate acts such as insulting others (Kurlan et al., 1996). These characteristic behaviours may all represent a failure to inhibit inappropriate behaviour in a social context. TS has been linked to disruption of fronto-striatal pathways (e.g., Robertson, 2000; Moriarty et al., 1997). Evidence has suggested basal ganglia abnormalities in both children and adults with TS (e.g., Hyde et al., 1995; Peterson et al., 1993), with evidence of a shortened cortical silent period which may result from inadequate inhibition of basal ganglia pathways to motor cortex (Ziemann, Paulus, & Rothenberger, 1997). In addition, there is some evidence for frontal lobe abnormalities in both children and adults with TS, particularly in lateral and anterior cingulate areas (Petersen et al., 2001; Eidelberg et al., 1997; Chase, Geoffrey, Gillespie, & Burrows, 1986; but see also Weeks, Turjanksi, & Brooks, 1996).

Early studies of cognitive functioning in adults with TS found evidence of executive deficits (e.g., Channon, Flynn, & Robertson, 1992; Bornstein, 1991). However, these studies did not screen participants adequately for comorbid symptomatology such as obsessive compulsive disorder (OCD) and attention deficit hyperactivity disorder (ADHD), both of which are associated independently with impairments on executive tasks (e.g., Shallice et al., 2002; Cavedini, Ferri, Scarone, & Bellodi, 1998). Studies of children and adolescents with TS have reported mixed findings. The most parsimonious view, put forward by some authors, is that any cognitive deficits seen in TS are likely to be attributable to comorbid conditions (e.g., Yeates & Bornstein, 1994; Ozonoff, 1997). Alternatively, others have suggested that TS might be characterised by a selective deficit in behavioural inhibition (e.g., Channon, Pratt, & Robertson, 2003a; Baron-Cohen, Cross, Crowson, & Robertson, 1994). However, this merits further investigation since inhibition is probably not a

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unitary construct, and there may be many separable types of inhibition, all with different neural substrates (e.g., Kok, 1999; Nigg, 2000). It is important therefore to take into consideration the particular tasks used to assess inhibition.

Considering only controlled studies that have excluded participants with comorbid diagnoses, impairment has been reported in studies of both adults (Channon, Crawford, Vakili, & Robertson, 2003b) and children (Channon et al., 2003a) on a sentence completion measure involving both inhibition and strategy generation. By contrast, no inhibitory impairment has been reported in children with uncomplicated TS using the Stroop test (Ozonoff & Jensen, 1999) or a negative priming task (Ozonoff, Strayer, McMahon, & Filloux, 1998). With respect to other executive functions, studies have generally found no deficits in children with uncomplicated TS on tasks involving working memory, planning and set-shifting such as the Wisconsin Card Sorting Test (WCST) (Channon et al., 2003a, Ozonoff & Jensen, 1999; Schuerholz, Baumgardner, Singer, Reiss, & Denkla, 1996) or Tower of Hanoi (Ozonoff & Jensen, 1999). Similarly, adults with uncomplicated TS have been reported to have no deficits on the Trail-Making Test (Channon et al., 2003b), although one study reported that adults with uncomplicated TS were impaired on executive aspects of memory, including strategic free recall and working memory tasks (Stebbins et al., 1995).

Most studies of executive functioning in TS have used traditional clinical measures such as the WCST. However, such tasks typically involve multiple executive and non-executive processes, often lack control conditions, and may be failed for many different reasons. The current study investigated the inhibitory deficit hypothesis of uncomplicated TS using experimental measures linked with different areas within the frontal lobes. Since nonsensical sentence completion has proved to be sensitive in previous studies of uncomplicated TS, this was included as one of the measures involving inhibition. To study the inhibitory processes in more detail, inhibition of both high and low prepotent sentence completion endings was manipulated directly. Since sentence completion is not a pure inhibitory measure, the Flanker task (Eriksen & Eriksen, 1974) was also included. This assesses inhibition of interference by comparing conflicting trials (flanker arrows pointing in different directions from central arrows) with compatible trials (all arrows pointing in the same direction). The Flanker task has similarities to the Stroop task, but is not influenced by reading difficulties or colour blindness. Non-inhibitory tasks assessing working memory and reward learning were also included. It was predicted that the TS group would perform more poorly than the control group on the inhibitory tasks but not on the working memory and reward learning tasks, consistent with the inhibitory deficit hypothesis.

Methods

Participants and procedure

Twenty participants (13 m, 7f) who met DSM-IV^{TR} criteria (American Psychiatric Association, 2000) for TS took part in the study. Diagnosis of TS was established by a clinician with extensive experience in the diagnosis of TS and related disorders (MMR), using an updated version of the National Hospital Interview Schedule for Gilles de la Tourette Syndrome (Robertson & Eapen, 1996). This instrument was designed specifically for TS research, and is widely used in the UK. The original study showed that it had good inter-rater reliability and also good concurrent validity when compared with another widely used instrument, the Yale Schedule. A further twenty participants made up the control group (13 m, 7f). Exclusion criteria included a diagnosis of neurological disorder (e.g., head injury) or major psychiatric illness (other than TS). All participants were screened for comorbid psychiatric disorder, including ADHD, OCD, depression, anxiety disorders and psychosis, using the Structured Clinical Interview for DSM-IV (SCID; First, Spitzer, Gibbon, & Williams, 1996). Participants were also excluded if their scores on the Raven's Standard Progressive Matrices (Raven's SPM; Raven, 1960) fell below the 5th percentile for their age group. All participants included in the study were between 11 and 18 years old, and fluent in English. The TS and control groups were matched for age ($t = .20$, $df = 38$, $p = .841$), and IQ, as measured by age-related percentiles on the Raven's SPM ($t = .23$, $df = 38$, $p = .822$); mean scores and standard deviations are shown in Table 1. The order of presentation of the experimental tasks was counterbalanced within each group.

In addition to the clinical ratings described above, several self-report measures of symptomatology were administered. The TS participants were given The Yale Global Tic Severity Rating Scale (Leckman et al., 1989) as a measure of their current TS symptomatology. ADHD symptomatology was measured using the Brown Attention-Deficit Disorder Scale for children (Brown ADD Scale; Brown, 1996), and OCD symptomatology was measured using the Leyton Obsessional Inventory–Child Version (LOI-CV; Berg, Rapoport, & Flament,

Table 1 Mean scores and standard deviations for age, IQ and measures of symptomatology

	TS group		Control group		
	Mean	(sd)	Mean	(sd)	*p*
Age	14.40	(2.44)	14.25	(2.27)	.841
IQ (age-related percentiles)	55.85	(31.15)	53.70	(28.68)	.822
				Gp × rater	.038*
				Rater	.054
DEX: Self-rated	19.20	(12.81)	17.75	(13.22)	.727
DEX: Other-rated	19.20	(13.74)	8.45	(5.91)	.003**
Leyton Obsessional Inventory	5.55	(6.19)	4.00	(6.25)	.442
Brown Scale for ADHD	37.95	(25.85)	37.90	(23.46)	.995
Yale Tic Severity	21.20	(17.97)			

**$p < .01$; *$p < .05$.

1986; Berg, Whitaker, Davies, Flament, & Rapoport, 1988). *T*-tests were carried out to compare the two groups on the symptomatology scores. The TS and control groups did not differ significantly on either their LOI-CV scores ($t = .78$, $df = 37$, $p = .442$) or their Brown ADD Scale scores ($t = .01$, $df = 37$, $p = .995$); mean scores and standard deviations are shown in Table 1.

The DEX (Dysexecutive Questionnaire; Wilson, Alderman, Burgess, Emslie, & Evans, 1996) provides information on dysexecutive behaviours in daily life, including emotion, personality, motivation, behaviour and cognition. It is intended for use both as a self-rating measure, and for ratings by others who know the participant well. Participants completed the questionnaire themselves (self-ratings), and a relative or teacher who knew them well completed an equivalent questionnaire (independent-ratings). Comparison of the DEX ratings was made using ANOVA, which showed that the group by rater interaction ($F = 4.63$, $df = 1,38$, $p = .038$) was significant; the main effect of group did not reach significance ($F = 3.96$, $df = 1,38$, $p = .054$). Post-hoc *t*-tests showed that the groups did not differ significantly in their self-ratings ($t = .35$, $df = 38$, $p = .727$), but that the TS participants were judged by others to show significantly more dysexecutive behaviours than the control participants ($t = 3.21$, $df = 38$, $p = .003$). Mean scores are shown in Table 1.

Of the twenty participants in the TS group, five (25%) were taking prescribed medication for management of their TS symptoms at the time of testing. Three were taking sulpiride, one was taking haloperidol and one was taking clonidine.

Experimental measures

Inhibition. With respect to measures involving inhibition, imaging studies have indicated that the anterior cingulate and ventrolateral prefrontal cortex are activated during tasks involving suppression of a prepotent response such as the Hayling and Stroop Tests (Taylor, Kornblum, Lauber, Minoshima, & Koeppe, 1997; Nathaniel-James, Fletcher, & Frith, 1997; Pardo, Pardo, Janer, & Raichle, 1990), and that an increase in anterior cingulate activation is associated with increased amount of conflict in a Stroop task (Carter et al., 2000). Similarly, a study using the Flanker test (Eriksen & Eriksen, 1974), found that anterior cingulate activation was associated with stimulus–response conflict (Botvinick, Nystroom, Fissell, Carter, & Cohen, 1999).

(a) *Flanker test.* The Flanker test used in the present study was based on that used by Botvinick et al. (1999). The display consisted of five arrows (one central arrow and two 'flankers' to either side). Participants were instructed to press one of two response keys ('left' or 'right'), as quickly as possible, in accordance with the direction of the central arrow. Thus, if the central arrow pointed to the left, they were required to press the key on the left, and vice versa. The four 'flanker' arrows all pointed in either the same direction as the central arrow (compatible) or in the opposite direction (incompatible). Each participant received 2 practice blocks and 4 experimental blocks of 40 trials. Each block contained 20 incompatible and 20 compatible trials (10 of each of the 4 possible trial types). Each display remained on the screen until the participant pressed a response key. The inter-trial interval (ITI) was 1.2 seconds. The order of presentation was pseudo-randomised within each block, such that there were never more than 4 consecutive identical correct responses (left or right key), and never more than 2 consecutive identical trial types. For each participant the percentage of correct responses and median reaction times (RT) were recorded for compatible and incompatible trials.

(b) *Sentence Completion test.* This task was based on the Hayling Test developed by Burgess and Shallice (1996, 1997). It involves completing sentences as quickly as possible with words that make sense (part A) and with nonsensical words (part B). The sentences used in the Hayling test were taken from a study described by Bloom and Fischler (1980), which gave the frequencies of responses for the words used by college students when asked to complete sentences with a missing last word. Since the Hayling test did not examine the effect of prepotency on performance, the current task examined this by selecting two sets of sentences from the Bloom and Fischler set, those of high and low prepotency. Those with high prepotency were sentences for which there was one highly frequent response given by the sample, making them potentially harder responses to inhibit. For example, when asked to provide the last word to the sentence 'The children went outside to_____', 99% of the sample responded 'play'. The low prepotent sentences were those for which a variety of responses were provided, with no answer having a markedly higher frequency than any other answer. For example, when presented with the sentence 'The sun went down before we could_____', the most frequent responses were 'leave' and 'see' with 18% each.

When the sentences had been selected they were allocated to two sets, I and II, each containing 15 high prepotent and 15 low prepotent sentences. Half of the participants received set I for part A of the task and the other half received set II for part A. Within each set, two random orders of sentence presentation were created to mix high and low prepotency sentences. The sentences were presented orally by computer. The experimenter recorded median RT by pressing a response key when the participant responded, and then recording the word given on a response sheet. Although voice activation recording is generally used to time oral responses accurately, it was thought that vocal tics would potentially render this problematic.

The quality of participants' nonsensical completions in part B of the test was rated following similar principles to those outlined by Burgess and Shallice (1996). Straightforward, obvious completions scored 3, while responses that were not obvious completions, but were nevertheless related to the context of the sentence, scored 1; scores for straightforward and related completions were added to give separate error scores for the high and low prepotency sentences.

Working memory: N-back test. The N-back test (Braver et al., 1997) is a measure of working memory that allows comparison of performance across subtasks in which the memory load gets progressively larger. Functional imaging studies have associated perform-

ance on this task with activation in the dorsolateral prefrontal cortex (Braver et al., 2001; Callicott et al., 1999), and it has been shown that activation in dorsolateral areas increases as the memory load increases (Cohen et al., 1997).

The N-back test used in the present study compared performance on three conditions, with increasing working memory load. All conditions required participants to press one of two response keys ('yes' or 'no') as quickly as they could, in response to letters presented individually on the computer screen. In the 0-back condition, they were asked to press 'yes' to one target letter (B), and 'no' to any other letter; three other letters were used as distracters. In the 1-back condition, participants were required to press the 'yes' key whenever the letter on the screen was identical to the immediately preceding letter. In the 2-back condition, they were asked to press the 'yes' key whenever the letter on the screen was the same as the letter presented two trials previously. The design of the test was similar to the Flanker test, in that each task consisted of 2 practice blocks and 4 experimental blocks, with an ITI of 1.2 seconds. The blocks consisted of 40 trials each for the 0-back condition, 41 trials for the 1-back condition and 42 trials for the 2-back condition. The first trial of each block for the 1-back condition and the first two trials of each block for the 2-back condition were excluded from the analysis, since the response was automatically 'no'. The three tasks were matched such that 25% of the letters within each block were targets, and 75% were distracters. The order of the 0-back, 1-back and 2-back tasks was counterbalanced within each group. For each participant the percentage of correct responses and median RT were recorded for each condition.

Reward learning: Gambling test. Reward learning tasks involve learning responses based on achieving reward and/or avoiding punishment. The orbitofrontal cortex is thought to be involved in controlling and modifying reward-related behaviour (Rolls, 1999). Recent studies have examined reward learning using 'Gambling' tasks, and patients with lesions involving orbitofrontal and medial frontal lesions have been shown to be impaired relative to those with dorsolateral lesions (Rogers et al., 1999a; Bechara, Damasio, Tranel, & Anderson, 1998). A functional imaging study has shown that performance on Gambling tasks is associated with orbitofrontal but not dorsolateral activation (Rogers et al., 1999b). However, recent work has suggested that more extensive lesions may be needed to impair performance substantially (Manes et al., 2002).

The Gambling test used in the current study was described by Bechara, Tranel, and Damasio (2000). Participants were shown four decks of cards on a computer screen, labelled 'A', 'B', 'C' and 'D'. Each of the 4 decks contained 20 red and 20 black cards, although the colour of the cards did not affect their value. Overall, decks A and B were 'bad' and decks C and D were 'good'. Decks A and B were associated with higher gains than decks C and D, but even higher long-term losses. Both rewards and punishments varied within each deck. The first 10 cards of decks A and B totalled an overall reward of £1,000, and an overall punishment of £1,250. For each subsequent 10 cards in these two decks, the amount of reward increased overall by £100 whilst the amount of punishment increased by £250. For decks C and D, the first 10 cards totalled an overall reward of £500, and an overall punishment of £250. For each subsequent 10 cards in decks C and D the amount of reward increased by £50, while the amount of punishment increased by £25. Decks A and C gave small, frequent punishments, whereas Decks B and D gave large, infrequent punishments. Participants were asked to select a card on each trial, with the aim of winning as much money as possible. Every card selection resulted in winning some money, but on some trials, participants also lost money; gains and losses were indicated on the screen. The task stopped automatically when the participant had made 100 card selections. Percentage of choices from the 'bad' decks and median RT for each block of 20 cards was calculated for each participant.

Results

In order to deal with possible effects of age and IQ, analysis of covariance (ANCOVA) was carried out to compare the groups on the experimental tasks, using age and Raven's percentile scores as covariates. Since each of the experimental tasks contained measures of both accuracy and speed, a significance level of alpha = .05/2 ($p = .025$) was adopted. Since median RT was measured for each participant, mean scores reported for each group for speed refer to mean of median RT. The mean scores and standard deviations for each test are shown in Table 2 for both groups.

Inhibition: (a) Flanker test

Accuracy. ANCOVA was used to compare the groups on the compatible and incompatible trials, using one between-groups factor (group: TS or control) and one within-groups factor (type of trial: compatible or incompatible). This showed a significant group by trial type interaction ($F = 6.30$, df = 1,36, $p = .017$). The effect of group did not reach significance ($F = 3.46$, df = 1,36, $p = .071$). Post-hoc ANCOVAs for compatible and incompatible trials separately showed that the TS group performed significantly less accurately than the control group on the incompatible trials ($F = 6.55$, df = 1,36, $p = .015$), but that the groups did not differ significantly on the compatible trials ($F = .45$, df = 1,36, $p = .509$).

Speed. Logarithmic transformations were carried out on the data for the two groups. ANCOVA using one between-groups factor (group: TS or control) and one within-groups factor (type of trial: compatible or incompatible) showed a significant group by type of trial interaction ($F = 7.31$, df = 1,36, $p = .010$). There was no significant main effect of group ($F = 1.61$, df = 1,36, $p = .213$). Mean scores showed that relative slowing on the incompatible versus compatible trials was greater for the TS group. They tended to perform faster in both conditions than the control group, but post-hoc ANCOVAs examining

Table 2 Mean scores and standard deviations for the experimental tasks

	TS group		Control group		
	Mean	(SD)	Mean	(SD)	*p*
Flanker test					
Accuracy (% correct trials)					
					Gp × trial type .017*
					Gp .071
Compatible	96.38	(4.60)	97.00	(3.23)	.509
Incompatible	92.94	(5.89)	96.25	(2.87)	.015*
Speed (secs per item)					
					Gp × trial type .010*
					Gp .213
Compatible	.47	(.10)	.51	(.10)	.164
Incompatible	.49	(.10)	.52	(.10)	.343
Sentence Completion test					
Accuracy (error scores)					
					Gp × prepotency .538
					Gp .021*
High prepotent sentences	3.25	(2.90)	1.80	(1.32)	
Low prepotent sentences	3.70	(3.63)	1.75	(1.65)	
Speed (secs per item)					
					Gp × condition × prepotency .617
					Gp × condition .936
					Gp × prepotency .074
					Gp .024*
Sensible completions:					
High prepotent sentences	1.09	(.26)	.85	(.14)	
Low prepotent sentences	2.32	(1.45)	1.49	(.50)	
Nonsensical completions:					
High prepotent sentences	2.58	(1.91)	1.85	(1.13)	
Low prepotent sentences	3.31	(3.06)	1.94	(1.12)	
N-back test					
Accuracy (% correct trials)					
					Gp × task .630
					Gp .108
0-back	95.47	(3.52)	96.16	(3.21)	
1-back	94.34	(4.26)	95.31	(3.13)	
2-back	83.50	(6.62)	85.94	(7.31)	
Speed (secs per item)					
					Gp × task .498
					Gp .129
0-back	.45	(.10)	.47	(.09)	
1-back	.53	(.12)	.58	(.17)	
2-back	.87	(.38)	1.06	(.54)	
Gambling test					
Accuracy (% cards from the 'bad' decks)					
					Gp × time .427
					Gp .790
Block 1	56.25	(7.05)	55.50	(14.59)	
Block 2	51.75	(11.15)	46.00	(13.04)	
Block 3	47.50	(17.95)	43.00	(16.17)	
Block 4	46.50	(20.72)	44.50	(20.58)	
Block 5	41.75	(24.67)	50.50	(19.53)	
Speed (secs per item)					
					Gp × time .740
					Gp .474
Block 1	.94	(.40)	1.10	(.54)	
Block 2	.75	(.31)	.96	(.67)	
Block 3	.76	(.36)	.95	(.70)	
Block 4	.77	(.34)	.78	(.34)	
Block 5	.71	(.37)	.81	(.41)	

*$p < .025$.

compatible and incompatible trials separately showed that the group differences did not reach significance for either the incompatible trials alone ($F = .92$, df = 1,36, $p = .343$) or the compatible trials alone ($F = 2.01$, df = 1,36, $p = .164$).

Speed–accuracy trade-off. In order to examine possible speed–accuracy trade-offs, Pearson correlations were performed for each group for the compatible and incompatible trials. This showed a near-significant correlation (using a strict

significance level of .025) for the TS group for the incompatible trials ($r = .46$, $p = .041$), such that faster RT was associated with lower accuracy; the correlation was not significant for compatible trials ($r = .18$, $p = .455$); nor did the control group show significant correlations for incompatible ($r = -.23$, $p = .328$) or compatible trials ($r = .05$, $p = .852$).

Inhibition: (b) Sentence Completion test

Accuracy. ANCOVA was used to compare the groups on the high and low prepotency sentences for the nonsensical completions, using one between-groups factor (group: TS or control) and one within-groups factor (prepotency: high or low). This showed no significant group by prepotency interaction ($F = .39$, $df = 1,36$, $p = .538$); the main effect of group was significant ($F = 5.82$, $df = 1,38$, $p = .021$). Examination of the mean scores showed that the TS group had higher error scores than the control group for both high and low prepotency trials.

Speed. Logarithmic transformations were carried out on the data for the two groups. ANCOVA was used to examine the speed of the two groups across both sensible and nonsensical completion conditions, using one between-groups factor (group: TS or control) and two within-groups factors (prepotency: high or low; condition: sensible or nonsensical). The group by condition by prepotency interaction was not significant ($F = .26$, $df = 1,36$, $p = .617$); nor was there a significant group by condition ($F = .01$, $df = 1,36$, $p = .936$) or group by prepotency interaction ($F = 3.40$, $df = 1,36$, $p = .074$). The main effect of group was significant ($F = 5.52$, $df = 1,38$, $p = .024$). Mean scores showed that the TS group performed more slowly than the control group on the tasks.

Speed–accuracy trade-off. In order to examine possible speed-accuracy trade-offs, Pearson correlations were performed for each group for the nonsensical completions. This showed a significant correlation (using a strict significance level of .025) for the TS group ($r = .52$, $p = .018$), such that slower RT was associated with higher error scores; the correlation was not significant for the control group ($r = .36$, $p = .119$). However, Fisher's (1921) test for the difference between two independent correlations showed that the correlation for the TS group did not differ significantly from the correlation for the control group ($z = .58$, $p > .05$).

Working memory: N-back test

Accuracy. ANCOVA using one between-groups factor (group: TS or control) and one within-groups factor (task: 0, 1, or 2-back) showed no significant group by task interaction ($F = .47$, $df = 2,35$, $p = .630$), nor a significant main effect of group ($F = 2.71$, $df = 1,36$, $p = .108$).

Speed. Logarithmic transformations were carried out on the data for the two groups. ANCOVA using one between-groups factor (group: TS or control) and one within-groups factor (task: 0, 1, or 2-back) showed no significant group by task interaction ($F = .71$, $df = 2,35$, $p = .498$); nor was there a significant main effect of group ($F = 2.41$, $df = 1,36$, $p = .129$).

Reward learning: Gambling test

Accuracy. ANCOVA was used to examine whether there were any changes in the percentage of cards selected from 'bad' decks by the two groups over time, using one between groups factor (group: TS or control) and one within-groups factor (time: 1, 2, 3, 4 or 5). The results showed that there was no significant group by time interaction ($F = .99$, $df = 4,33$, $p = .427$), nor a significant main effect of group ($F = .07$, $df = 1,36$, $p = .790$).

Speed. ANCOVA using logarithmically transformed data was used to examine whether there were any changes in the speed of performance by the two groups over time, using one between-groups factor (group: TS or control) and one-within groups factor (time: 1, 2, 3, 4, or 5). The results showed that there was no significant group by time interaction ($F = .49$, $df = 4,33$, $p = .740$), nor a significant main effect of group ($F = .09$, $df = 1,36$, $p = .474$).

Relationship to symptomatology scores and effects of medication

In order to examine any correlations between performance on the experimental tasks and symptomatology scores for the Yale Global Tic Severity Rating Scale, the Brown ADD Scale, the LOI-CV and the DEX questionnaire for the TS group, Spearman rank correlations were calculated (see Table 3). Separate correlations were carried out for the compatible and incompatible trials of the Flanker test, which showed significant interactions with group; composite scores were used for the other three tasks, where no significant interactions were found. The composite scores for the Sentence Completion test were total error score on Part B of the task, median speed on Part A, and median speed on Part B. For the N-back test, the percentage of correct responses and RT were averaged across the three conditions (0-, 1- and 2-back). For the Gambling test, the composite scores were the overall percentage of choices from the 'bad' decks and the median RT across all 100 card selections. For all tasks, logarithmic transformations were carried out on the composite speed measures prior to carrying out the correlations. No significant correlations were found with symptomatology scores for the Sentence Completion or Gambling tasks. For the incompatible trials of the Flanker task, accuracy correlated significantly with LOI-CV, while speed correlated significantly with Yale scores. For compatible trials,

Table 3 Correlations between symptomatology scores and experimental task performance for the TS group

Experimental task	Yale tic severity score	Brown ADD	LOI-CV Scale score	DEX Self-rated	DEX Independent-rated
Flanker:					
Incompatible trials:					
Accuracy	$r = -.049$	$r = -.440$	$r = -.446$	$r = -.430$	$r = -.107$
	$p = .836$	$p = .060$	$p = .049$*	$p = .058$	$p = .654$
Speed	$r = .512$	$r = .008$	$r = -.186$	$r = -.263$	$r = .078$
	$p = .021$*	$p = .974$	$p = .432$	$p = .262$	$p = .745$
Compatible trials:					
Accuracy	$r = -.240$	$r = -.588$	$r = -.597$	$r = -.613$	$r = -.449$
	$p = .309$	$p = .008$**	$p = .005$**	$p = .004$**	$p = .047$*
Speed	$r = .472$	$r = .083$	$r = -.125$	$r = -.152$	$r = .130$
	$p = .035$*	$p = .734$	$p = .599$	$p = .522$	$p = .584$
Sentence Completion:					
Error score	$r = .095$	$r = -.074$	$r = -.112$	$r = -.208$	$r = -.047$
	$p = .690$	$p = .764$	$p = .639$	$p = .378$	$p = .845$
Speed Part A	$r = -.127$	$r = -.212$	$r = -.098$	$r = -.135$	$r = -.066$
	$p = .593$	$p = .383$	$p = .682$	$p = .569$	$p = .781$
Speed Part B	$r = .102$	$r = -.269$	$r = -.224$	$r = -.273$	$r = -.383$
	$p = .670$	$p = .266$	$p = .342$	$p = .244$	$p = .096$
N-back:					
Accuracy	$r = -.176$	$r = -.608$	$r = -.673$	$r = -.480$	$r = -.496$
	$p = .457$	$p = .006$**	$p = .001$**	$p = .032$*	$p = .026$*
Speed	$r = .183$	$r = -.106$	$r = -.392$	$r = -.225$	$r = -.108$
	$p = .440$	$p = .665$	$p = .087$	$p = .340$	$p = .649$
Gambling:					
Accuracy	$r = .039$	$r = -.212$	$r = -.270$	$r = -.404$	$r = .082$
	$p = .872$	$p = .384$	$p = .250$	$p = .077$	$p = .732$
Speed	$r = -.286$	$r = .104$	$r = .085$	$r = .180$	$r = .182$
	$p = .221$	$p = .670$	$p = .720$	$p = .448$	$p = .442$

*$p < .05$; **$p < .01$.

accuracy correlated significantly with Brown ADD, LOI-CV, DEX self- and independent-ratings, while speed correlated significantly with Yale scores. For the N-back task, overall accuracy correlated significantly with Brown ADD, LOI-CV, DEX self- and independent-ratings. All significant correlations were in the expected direction, i.e., higher symptomatology correlated with poorer performance.

The above correlations showed some relationships between cognitive performance and symptomatology, although this was more apparent for the measures that did not differentiate the groups. To investigate this further, group comparisons on the experimental tasks were repeated using ANCOVA with the symptomatology scores as additional covariates. This made no difference to the pattern of findings for any of the tasks.

The effects of medication on performance were examined within the TS group for the Sentence Completion and Flanker measures that differentiated the groups. Analyses showed no significant differences between the medicated and unmedicated participants on these measures, nor any significant task by medication effects ($p > .05$).

Discussion

As expected, the groups did not differ significantly on measures of working memory (N-back test) or reward learning (Gambling test). Predictions regarding inhibitory deficits were partially confirmed since the participants with TS performed more poorly than a matched control group on an inhibitory sentence completion measure, and were less accurate on another inhibitory measure, the Flanker test. However, Sentence Completion did not show the expected interaction with prepotency or type of task. The expected inhibitory interaction was found for accuracy on the Flanker test, but was less clear cut for speed. With respect to everyday functioning, independent- but not self-ratings on the DEX questionnaire showed the TS group to have more dysexecutive behaviours than the control group; this is similar to the pattern of findings reported by Channon et al. (2003b) with adult TS participants. There was little evidence of significant correlations between DEX scores and inhibitory task performance. There was also little evidence that prescribed medication for the TS group influenced the findings, but the sample sizes were too small to draw firm conclusions about this.

Another potentially confounding factor in TS research is the question of comorbid symptomatology, especially ADHD and OCD, which may contribute to any executive deficits. For instance, Shallice et al. (2002) reported that children with ADHD were impaired on a range of tasks including the N-back, Sentence Completion and the Stroop task. The present study dealt with the question of comorbidity both categorically by including participants who did

not meet DSM criteria for comorbid disorders, and also dimensionally by measuring symptomatology directly. The TS group did not differ from the controls on the symptomatology measures, and any significant correlations between these and cognitive tasks were more often with non-inhibitory measures (i.e., Gambling, N-back and compatible Flanker trials) than with inhibitory measures (i.e., Sentence Completion, Flanker incompatible trials), affecting accuracy but not speed. Taken together, the findings suggest that comorbidity played at most a small role in any inhibitory difficulties shown by the TS group. With respect to Yale tic symptomatology, significant correlations were found only with inhibitory and non-inhibitory Flanker speed measures, and no relationships were found with task accuracy. Tics may potentially affect control of muscles involved in task performance (Ziemann et al., 1997; Moll et al., 2001). Since vocal responses were used for the Sentence Completion task, and finger responses for the flanker task, this is a potentially relevant factor, but does not explain the lack of group differences on the other experimental tasks, which also involved finger responses. Moreover, the current TS participants had relatively mild pathology. However, tic occurrence should be ideally measured during testing to assess this adequately.

What other factors could account for the pattern of findings on the experimental tasks? Age and IQ differences do not readily explain group differences, since the groups were matched on these, and they were also used as covariates. There was no reason to suspect that poorer motivation, attention or differential fatigue for the TS group on the inhibitory tests, since the groups did not differ on the other experimental measures and order of tasks was counterbalanced. It is difficult to argue that the inhibitory tasks were harder than the N-back or Gambling tests; the TS participants did not differ from the control group regardless of working memory load in the N-back test. Cognitive processes less reliant on executive skills such as language comprehension, long-term memory or visuospatial skills were not assessed directly in the present study, but there was no indication that the memory or visuospatial demands were any greater for the inhibitory tasks. Another possible explanation that can be rejected is overall psychomotor speed, since the TS group was differentially slowed only on the Sentence Completion test. There was no evidence that the TS participants used a different strategy from the control group in prioritising either speed or accuracy on the Sentence Completion test, since they performed more poorly on both, although a speed–accuracy trade-off may have contributed to the pattern of TS performance on the Flanker test.

Consistent with the inhibitory deficit hypothesis, the TS group was not impaired on the N-back and Gambling tasks for either speed or accuracy. With respect to inhibitory performance, the Flanker task was expected to show impairment on incompatible but not compatible trials. This pattern emerged for accuracy, but was less clear cut for speed since the group difference did not reach significance for the incompatible trials, and the TS mean RTs were slightly faster than the control mean RTs. Moreover, correlations suggested a speed–accuracy trade-off for the incompatible trials for the TS group (but not the control group). This may indicate impulsive responding in some individuals with TS, since less accurate performance tended to be associated with faster RTs. For the Sentence Completion task, the TS participants were in line with expectations based on an inhibitory deficit in that they made more errors in completing sentences with nonsensical endings than the control group. This may reflect the disruption of verbal or conceptual processes involved in task performance, rather than pure inhibitory processes. Participants may have failed to develop the strategies typically used by normal participants to increase efficiency, such as naming objects in the room or drawing words from semantic categories (Burgess & Shallice, 1996). Strategy use was not assessed directly, but could potentially explain higher error scores and slowed performance. However, the findings with respect to speed were not consistent with expectations, since they showed non-specific slowing across both the sensible and nonsensical completion conditions. Burgess and Shallice (1996) speculated that slower speed for sensible completions reflected initiation difficulties. If this applies in the present case, the concept of an inhibitory deficit may not be adequate to account for TS performance.

Inhibition is not necessarily a unitary construct. Distinctions have been postulated between automatic (passive) and effortful (active) inhibition (Kok, 1999; Nigg, 2000). It has also been suggested that there may be a range of potentially independent inhibitory processes within these categories. For example, passive paradigms such as orienting responses, habituation and prepulse inhibition may be separable (Kok, 1999). Similarly, effortful tasks such as those involving interference control, cognitive inhibition, behavioural inhibition and oculomotor inhibition may be measuring separable inhibitory processes with different underlying neural substrates (Nigg, 2000). It is possible that cognitive impairments in uncomplicated TS are linked to very specific types of inhibition, and future studies could examine this further by using measures postulated to relate to these potentially separable processes.

In summary, the findings provided further evidence that uncomplicated TS is not associated with widespread executive impairments. However, it was not clear that any group differences could be attributed solely to selective inhibitory impairment. Mild deficits in uncomplicated TS may be detected only by sensitive measures, contributing to the difficulties of determining the precise nature of performance difficulties. Comorbid symptomatology

is common in TS, and is likely to be associated with more severe and/or more extensive executive impairment than that shown by the present sample of individuals with a pure disorder. A broader issue for future research is the extent to which neuropsychiatric disorders have unique or shared patterns of cognitive deficits.

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Tourette's Syndrome (TS): Cognitive Performance in Adults With Uncomplicated TS

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Tourette's syndrome (TS) is a neurodevelopmental disorder associated with frontostriatal dysfunction. The extent of any cognitive impairment associated with uncomplicated TS is unclear, as comorbid psychiatric symptomatology is thought to contribute to cognitive deficits. Previous studies have found evidence of mild performance deficits, most commonly on tasks that involve inhibitory processes. The present study evaluated this in carefully screened adult participants with TS. The findings showed the TS group to perform more poorly on one test involving behavioral inhibition (sentence completion), but did not provide strong support for an interpretation based solely on inhibitory deficits, and there was no evidence of impairment on another behavioral inhibition task (flanker test). There were also no differences between the groups on tasks involving working memory (*n*-back), task switching, or object alternation learning. The findings provide further evidence that uncomplicated TS is associated with only mild, circumscribed impairment. The nature of any impairment is discussed.

Keywords: Tourette's syndrome, executive functions, inhibition, obsessionality, ADHD

Tourette's syndrome (TS) is a neurodevelopmental disorder in which the core symptomatology consists of motor and vocal tics. It has been linked to a range of everyday problems including "disinhibited behaviors" (A. J. Cohen & Leckman, 1992), coprolalia, copropraxia, and social inappropriateness (Kurlan et al., 1996). Dopaminergic basal ganglia circuitry is thought to be implicated in TS, with disruption of frontostriatal pathways supporting motor and cognitive functioning (see, e.g., Chase, Geoffrey, Gillespie, & Burrows, 1986; Moriarty et al., 1997; Robertson, 2000), although others have suggested alternative models (see, e.g., Weeks, Turjanksi, & Brooks, 1996).

With respect to cognitive functioning, the evidence to date has been mixed. Early studies suggested executive deficits in adults with TS (e.g., Bornstein, 1991; Channon, Flynn, & Robertson, 1992) but did not carry out adequate screening to differentiate between impairment associated with TS and that linked to comorbid symptomatology. Obsessive–compulsive disorder (OCD) and attention-deficit/hyperactivity disorder (ADHD) commonly occur in conjunction with TS, and because these are known to be associated with executive deficits (e.g., Cavedini, Ferri, Scarone, & Bellodi, 1998; Shallice et al., 2002), presence of these disorders may account for any deficits in TS (e.g., Ozonoff, 1997; Yeates & Bornstein, 1994). Comparison of TS participants with and without ADHD/OCD symptomatology has tended to suggest more extensive impairment in those with comorbid disorder, both in children (e.g., Brand et al., 2002; Sherman, Shepard, Joschko, & Freedman, 1998) and in adults (e.g., Silverstein, Como, Palumbo, West, & Osborn, 1995), supporting the view that comorbidity plays a role. However, an account solely in these terms fails to explain the presence of cognitive impairment often found in uncomplicated TS after screening out those with comorbid disorders.

An alternative view is that uncomplicated TS may be characterized by relatively circumscribed cognitive impairment. As tasks sensitive to uncomplicated TS have often contained inhibitory components, it has been postulated that the central impairment in TS is inhibitory in nature (see, e.g., Baron-Cohen, Cross, Crowson, & Robertson, 1994; Channon, Pratt, & Robertson, 2003; Pennington & Ozonoff, 1996). However, inhibition is not a unitary construct, and there may be many separable types, all with different neural substrates (e.g., Kok, 1999; Nigg, 2000). Moreover, multiple cognitive factors typically contribute to neuropsychological tasks, including both inhibitory and other skills. The particular characteristics of the tasks used are therefore critical. The age of the participants with TS may also be a factor, as the symptoms of the disorder often lessen with increasing age (see, e.g., Robertson, 2000); there may be corresponding reductions in cognitive impairment. With respect to tasks involving inhibitory skills, the evidence relating to uncomplicated TS is inconsistent for the Stroop test (e.g., Ozonoff & Jensen, 1999), no inhibitory impairment has been reported in children on a negative priming task (Ozonoff, Strayer, McMahon, & Filloux, 1998), and marginal impairment was found in children on an inhibitory flanker test (Crawford, Channon, & Robertson, 2005). Impairment on a sentence completion measure involving both inhibition and strategy generation has been found in both children (Channon, Pratt, & Robertson, 2003; Crawford et al., 2005) and adults with uncomplicated TS (Channon, Crawford, Vakili, & Robertson, 2003). Altered inhibitory priming has been reported in both children and adults with un-

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complicated TS (Swerdlow, Magulac, Filion, & Zinner, 1996). With respect to other executive functions, studies have generally found no deficits in children with uncomplicated TS on tasks involving working memory, planning, and set-shifting (e.g., Channon, Pratt, & Robertson, 2003; Ozonoff & Jensen, 1999; Schuerholz, Baumgardner, Singer, Reiss, & Denckla, 1996). Similarly, adults with uncomplicated TS have been reported to have no deficits on the Trail-Making Test (Channon, Crawford, et al., 2003), although one study reported impairment on executive aspects of memory (Stebbins et al., 1995).

Studies of executive functioning in TS have often used traditional clinical measures that often lack control conditions, such as the Wisconsin Card Sorting Test. The current study investigated the inhibitory deficit hypothesis of uncomplicated TS using experimental measures linked with different areas within the frontal lobes. Because nonsensical sentence completion has proved to be sensitive in previous studies of uncomplicated TS, this was included as one of the measures involving inhibition. To study the inhibitory processes in more detail, inhibition of both high and low prepotent sentence completion endings was manipulated directly. As sentence completion is not a pure inhibitory measure, the flanker task (Eriksen & Eriksen, 1974) was also included. This assesses inhibition of interference by comparing conflicting trials (flanker arrows pointing in different directions from central arrows) with compatible trials (all arrows pointing in the same direction). The flanker task has similarities to the Stroop task but is not influenced by reading difficulties or color blindness. Noninhibitory tasks assessing working memory, task switching, and reward learning were also included. It was predicted that the TS group would perform more poorly than the control group on the inhibitory tasks but not on the other tasks, consistent with the inhibitory deficit hypothesis. Three of these tasks were previously studied in children with TS by Crawford et al. (2005); studying an adult group therefore permitted examination of whether any cognitive impairments were milder in an adult group.

Method

Participants and Procedure

Twenty participants (13 men, 7 women) who met *Diagnostic and Statistical Manual of Mental Disorders* (4th edition) criteria (DSM–IV; American Psychiatric Association, 2000) for TS took part in the study. Diagnosis of TS was established by a clinician with extensive experience in the diagnosis of TS and related disorders (MMR), using an updated version of the National Hospital Interview Schedule for Gilles de la Tourette Syndrome (Robertson & Eapen, 1996). This instrument was designed specifically for TS research and is widely used in the United Kingdom. The original study showed that it had good interrater reliability and also good concurrent validity when compared with another widely used instrument, the Yale Schedule (Pauls & Hurst, 1987). Twenty-five matched healthy participants made up the control group (16 men, 9 women). Exclusion criteria included a diagnosis of neurological disorder (e.g., head injury) or major psychiatric illness (other than TS). All participants were screened for comorbid psychiatric disorder including ADHD, OCD, depression, anxiety disorders, and psychosis using the Structured Clinical Interview for *DSM–IV* (SCID; First, Spitzer, Gibbon, & Williams, 1996). Those who met *DSM–IV* criteria for ADHD, OCD, or any other comorbid psychiatric disorder were excluded from the study, as were those with any history of learning disability, physical illness, or injury that might have affected brain function. Inclusion criteria were fluency in English, age between 18 and 50 years, and a Verbal IQ score of 80 or above on the National Adult Reading Test (Nelson, 1991).

In addition to the clinical ratings described above, several self-report measures of symptomatology were administered. The TS participants were given The Yale Global Tic Severity Rating Scale (Leckman et al., 1989) as a measure of their current TS symptomatology. ADHD symptomatology was measured using the Conners Adult ADHD Rating Scales—Short Version (CAARS; Conners, Erhardt, & Sparrow, 1998), and OCD symptomatology was measured using the Leyton Obsessional Inventory (LOI; Cooper, 1970) and the Maudsley Obsessive–Compulsive Inventory (MOCI; Hodgson & Rachman, 1977). *T* tests were carried out to compare the two groups on the symptomatology scores. The TS and control groups did not differ significantly in obsessionality on the LOI, $t(42) = 0.69$, $p = .493$, or the MOCI, $t(42) = 0.47$, $p = .639$, but did differ significantly in ADHD symptomatology on the CAARS, $t(41) = 2.47$, $p = .018$; mean scores and standard deviations are shown in Table 1.

Of the 20 participants in the TS group, 10 were taking prescribed medication for management of their TS symptoms at the time of testing. Nine of them were taking antipsychotic preparations, 3 of these in combination with an SSRI and 1 in combination with a tricyclic. The remaining participant was taking an SSRI alone.

Experimental Measures

With respect to measures involving inhibition, imaging studies have indicated that the anterior cingulate and ventrolateral prefrontal cortex are activated during tasks involving suppression of a prepotent response such as the Hayling and Stroop Tests (Nathaniel-James, Fletcher, & Frith, 1997; Pardo, Pardo, Janer, & Raichle, 1990; Taylor, Kornblum, Lauber, Minoshima, & Koeppe, 1997) and that an increase in anterior cingulate activation is associated with increased amount of conflict in a Stroop task (Carter et al., 2000). Similarly, a study using the flanker test (Eriksen & Eriksen, 1974), found that anterior cingulate activation was associated with stimulus-response conflict (Botvinick, Nystroom, Fissell, Carter, & Cohen, 1999).

Inhibition A: Sentence Completion Test

This task was based on the Hayling test developed by Burgess and Shallice (1996). It involves completing sentences as quickly as possible

Table 1
Mean Scores and Standard Deviations for the Demographic Measures

	TS ($n = 20$)		Control ($n = 25$)	
Variables	*M*	*SD*	*M*	*SD*
Demographic and IQ measures				
Age	31.05	11.32	28.88	7.97
Years of education	13.25	1.68	13.04	1.72
NART IQ	105.15	12.75	105.00	6.69
Clinical measures				
Conners ADHD Scale[a]	25.80	9.45	17.30	12.60
LOI[b]	15.11	10.51	12.68	12.24
MOCI[b]	5.11	4.20	4.52	3.96
Yale Tic Severity	27.00	19.43		

Note. TS = Tourette's syndrome; NART = National Adult Reading Test; ADHD = attention-deficit/hyperactivity disorder; LOI = Leyton Obsessional Inventory; MOCI = Maudsley Obsessive-Compulsive Inventory.
[a] Control $n = 23$. [b] TS $n = 19$.

with words that make sense (Part A) and with nonsensical words (Part B). The sentences used in the Hayling test were taken from a study described by Bloom and Fischler (1980), which gave the frequencies of responses for the words used by college students when asked to complete sentences with a missing last word. As the Hayling test did not examine the effect of prepotency on performance, the current task examined this by selecting two sets of sentences from the Bloom and Fischler set—those of high and low prepotency. Those with high prepotency were sentences for which there was one highly frequent response given by the sample, making them potentially harder responses to inhibit. For example, when asked to provide the last word to the sentence "The children went outside to _____," 99% of the sample responded "play." The low prepotent sentences were those for which a variety of responses were provided, with no answer having a markedly higher frequency than any other answer. For example, when presented with the sentence "The sun went down before we could _____," the most frequent responses were "leave" and "see," with 18% each.

When the sentences had been selected, they were allocated to two sets, 1 and 2, each containing 15 high prepotent and 15 low prepotent sentences. Half of the participants received Set 1 for Part A of the task, and the other half received Set 2 for Part A. Within each set, two random orders of sentence presentation were created to mix high and low prepotency sentences. The sentences were presented orally by computer. The experimenter recorded median reaction time (RT) by pressing a response key when the participant responded and then recording the word given on a response sheet. Although voice activation recording is generally used to time oral responses accurately, it was thought that vocal tics would potentially render this problematic.

The quality of participants' nonsensical completions in Part B of the test was rated following similar principles to those outlined by Burgess and Shallice (1996). Straightforward, obvious completions scored 3, whereas responses that were not obvious completions, but were nevertheless related to the context of the sentence, scored 1; scores for straightforward and related completions were added to give separate error scores for the high and low prepotency sentences.

Inhibition B: Flanker Test

This task was based on Eriksen and Eriksen (1974). It consisted of five arrows (one central arrow and two "flankers" to either side). On conflict trials, the central arrow and flanker arrows pointed opposite ways, requiring suppression of interference from the flanker arrows when responding to the central arrow. Participants were instructed to press one of two response keys ("left" or "right"), as quickly as possible, in accordance with the direction of the central arrow. Thus, if the central arrow pointed to the left, they were required to press the key on the left, and vice versa. The four "flanker" arrows all pointed in either the same direction as the central arrow (compatible) or in the opposite direction (incompatible). Each participant received two practice blocks and four experimental blocks of 40 trials. Each block contained 20 incompatible and 20 compatible trials (10 of each of the 4 possible trial types). Each display remained on the screen until the participant pressed a response key. The intertrial interval (ITI) was 1.2 s. The order of presentation was pseudorandomized within each block, such that there were never more than four consecutive identical correct responses (left or right key) and never more than two consecutive identical trial types. For each participant, the number of correct responses and median RTs for correct responses were recorded for compatible and incompatible trials.

Working Memory: N-Back Test

The *n*-back test (Braver et al., 1997) is a measure of working memory that allows comparison of performance across subtasks in which the memory load gets progressively larger. Functional imaging studies have associated performance on this task with activation in the dorsolateral prefrontal cortex (Braver et al., 2001; Callicott et al., 1999), and it has been shown that activation in dorsolateral areas increases as the memory load increases (J. D. Cohen et al., 1997).

The *n*-back test used in the present study compared performance on three conditions, with increasing working memory load. All conditions required participants to press one of two response keys ("yes" or "no") as quickly as they could, in response to letters presented individually on the computer screen. In the 0-back condition, they were asked to press "yes" to one target letter (*B*), and "no" to any other letter; three other letters were used as distractors. In the 1-back condition, participants were required to press the "yes" key whenever the letter on the screen was identical to the immediately preceding letter. In the 2-back condition, they were asked to press the "yes" key whenever the letter on the screen was the same as the letter presented two trials previously. The design of the test was similar to the flanker test, in that each task consisted of two practice blocks and four experimental blocks, with an ITI of 1.2 s. The blocks consisted of 40 trials each for the 0-back condition, 41 trials for the 1-back condition, and 42 trials for the 2-back condition. The first trial of each block for the 1-back condition and the first two trials of each block for the 2-back condition were excluded from the analysis, as the response was automatically "no." The three tasks were matched such that 25% of the letters within each block were targets and 75% were distractors. The order of the 0-back, 1-back, and 2-back tasks was counterbalanced within each group. For each participant, the percentage of correct responses and median RT were recorded for each condition.

Task Switching

Impairment on task switching paradigms has been reported in those with left prefrontal cortex lesions and with Parkinson's disease (e.g., Rogers et al., 1998), and imaging studies have shown dorsolateral prefrontal cortex involvement (e.g., Dove, Pollman, Schubert, Wiggins, & von Cramon, 2000). In this task, the correct response switched between letter and digit naming on every second trial in a predictable sequence, cued by the background color (e.g., blue for letters and yellow for digits). Like the flanker and *n*-back tasks, there were two practice blocks and four experimental blocks, with an ITI of 1.2 s. Two stimuli were presented on each trial, consisting of either a letter and a digit or one letter or digit, as appropriate, and one irrelevant symbol. Performance on switch trials was compared with nonswitch trials.

Object Alternation Learning

Object alternation learning has been found to be sensitive to perseverative responding after orbitofrontal and medial lesions in both primate and human studies (e.g., Freedman, Black, Ebert, & Binns, 1998; Mishkin, Vest, Waxler, & Rosvold, 1969). In this task, two objects (boxes) were presented on each trial and a reward was hidden under one of them. Either choice was rewarded on the first trial. After the first trial, the reward alternated from one object to the other after each correct response. When a correct choice was made, the box opened to display a coin. When an incorrect choice was made, the box opened to show nothing inside. Participants were instructed to choose the box containing the coin as often as possible. There were 50 trials in all, with an ITI of 5 s. For each participant, the number of total correct responses and median RT for correct responses was recorded.

Results

Experimental Measures

Because each of the experimental tasks contained measures of both accuracy and speed, we adopted a significance level of $\alpha = .05/2$ ($p = .025$). As median RT was measured for each partici-

pant, mean scores reported for each group for speed refer to mean of median RT, using logarithmically transformed data. The mean scores and standard deviations for each test are shown in Table 2 for each group; effect sizes are also shown.

Inhibition A: Sentence Completion Test

An analysis of variance (ANOVA) was used to compare the groups on error scores for the high and low prepotency sentences for the nonsensical completions, using one between-groups factor (group: TS or control) and one within-groups factor (prepotency: high or low). This showed no significant Group × Prepotency interaction, $F(1, 42) = 0.68$, $p = .415$, or main effect of prepotency, $F(1, 42) = 0.00$, $p = .983$. There was a significant main effect of group, $F(1, 42) = 6.27$, $p = .016$. Examination of the mean scores showed that the TS-alone group had higher error scores than the control group for both high and low prepotency trials.

An ANOVA was also used to examine the speed of the two groups across both sensible and nonsensical completion conditions, using one between-groups factor (group: TS or control) and two within-groups factors (prepotency: high or low; condition: sensible or nonsensical). The Group × Condition × Prepotency interaction was not significant, $F(1, 43) = 0.18$, $p = .672$, nor was there a significant Group × Condition, $F(1, 43) = 0.12$, $p = .733$, or Group × Prepotency interaction, $F(1, 43) = 1.71$, $p = .198$. The main effect of group was also not significant, $F(1, 43) = 0.34$, $p = .562$. There was a significant main effect of prepotency, $F(1, 43) = 91.17$, $p = .0001$, and of condition, $F(1, 43) = 56.63$, $p = .0001$.

Inhibition B: Flanker Test

An ANOVA was used to compare the groups on the compatible and incompatible trials, using one between-groups factor (group: TS or control) and one within-groups factor (inhibition: compatible or incompatible trials). This showed no significant Group × Inhibition interaction, $F(2, 42) = 0.23$, $p = .635$, or effect of group, $F(1, 43) = 1.67$, $p = .203$. There was a significant effect of inhibition, $F(1, 43) = 43.56$, $p = .0001$. For speed, the ANOVA showed no significant Group × Inhibition interaction, $F(1, 43) = 0.52$, $p = .474$, or effect of group, $F(1, 43) = 0.90$, $p = .766$. There was a significant main effect of inhibition, $F(1, 43) = 107.98$, $p = .0001$.

Table 2
Mean Scores, Standard Deviations, and Effect Sizes for the Cognitive Measures

Variables	TS (n = 20)		Control (n = 25)		Effect size
	M	*SD*	*M*	*SD*	
Sentence completion accuracy (errors)[a]*					
High prepotent nonsensical	4.16	3.78	2.64	2.25	0.24
Low prepotent nonsensical	4.58	3.50	2.24	2.62	0.35
Sentence completion speed per item in seconds					
High prepotent sensible	0.33	0.12	0.37	0.11	0.17
Low prepotent sensible	0.64	0.31	0.68	0.28	0.07
Sentence completion speed per item in seconds					
High prepotent nonsensical	0.76	0.38	0.82	0.39	0.04
Low prepotent nonsensical	1.06	0.76	0.94	0.54	0.09
Flanker percentage accuracy					
Compatible trials	98.19	2.52	98.95	1.93	0.17
Incompatible trials	95.88	2.90	96.95	3.00	0.18
Flanker speed per item in seconds					
Compatible trials	0.43	0.06	0.43	0.07	0.00
Incompatible trials	0.46	0.07	0.47	0.07	0.07
N-back percentage accuracy					
0-back	98.06	2.15	97.88	1.80	0.05
1-back	95.09	4.80	96.93	2.63	0.23
2-back	82.28	8.01	84.90	7.31	0.17
N-back speed per item in seconds					
0-back	0.43	0.10	0.46	0.20	0.09
1-back	0.59	0.45	0.58	0.29	0.01
2-back	1.01	0.49	1.12	0.48	0.11
Task switching percentage accuracy					
Switch trials	85.03	17.21	87.75	8.03	0.10
Nonswitch trials	86.42	17.16	90.50	6.39	0.16
Task switching speed per item in seconds					
Switch trials	0.87	0.24	0.96	0.32	0.16
Nonswitch trials	0.79	0.22	0.86	0.30	0.13
Object alternation					
Percentage of trials correct	63.80	18.33	67.76	14.63	0.12
Speed per item in seconds	0.72	0.29	0.83	0.44	0.15

Note. TS = Tourette's syndrome.
[a] TS n = 19.
* $p < .05$.

Working Memory: N-Back Test

On the *n*-back task, an ANOVA with one between-groups factor (group: TS or control) and one within-groups factor (working memory load: 0-, 1-, or 2-back) was used to compare accuracy of performance on the 0-back, 1-back, and 2-back conditions. There was no significant Group × Working Memory Load effect, $F(2, 42) = 2.00$, $p = .147$, and the effect of group was also not significant, $F(1, 43) = 1.85$, $p = .181$. The effect of memory load was significant, $F(2, 42) = 78.02$, $p = .0001$. For speed, the ANOVA showed no significant Group × Memory Load interaction, $F(2, 42) = 0.18$, $p = .835$, nor was there a significant main effect of group, $F(1, 43) = 0.58$, $p = .451$. The effect of memory load was significant, $F(2, 42) = 82.46$, $p = .0001$.

Task Switching

An ANOVA with one between-groups factor (group: TS or control) and one within-groups factor (switching: switch or nonswitch trials) was used to compare accuracy of task switching performance. There was no significant Group × Condition effect, $F(1, 43) = 1.02$, $p = .318$, and the effect of group was also not significant, $F(1, 43) = 0.83$, $p = .367$. The effect of switching was significant, $F(1, 43) = 9.55$, $p = .003$. For speed, the ANOVA showed no significant Group × Switching interaction, $F(1, 43) = 0.12$, $p = .730$, nor was there a significant main effect of group, $F(1, 43) = 1.21$, $p = .277$. The effect of switching was significant, $F(1, 43) = 31.55$, $p = .0001$.

Object Alternation Learning

On the alternation learning task, the ANOVA showed that the groups did not differ significantly in the number of trials correct, $t(43) = 0.81$, $p = .424$. Nor did the groups differ significantly in speed of performance, $t(43) = 1.01$, $p = .316$.

Relationship to Symptomatology Scores, Age, and Effects of Medication

Spearman rank correlations were calculated for the TS group to examine any correlations between performance on the experimental tasks and symptomatology scores on the Yale Global Tic Severity Rating Scale, CAARS, LOI, and MOCI (see Table 3).

Table 3
Correlations Between Cognitive Measures and Symptomatology Scores for the Group with Tourette's Syndrome

Cognitive measures	Yale Tic	CAARS	LOI	MOCI
Sentence completion accuracy (errors)[a]				
High prepotent nonsensical	.53*	.24	.30	.25
Low prepotent nonsensical	.51*	.06	.14	.16
Sentence completion speed per item in seconds				
High prepotent sensible	.04	−.11	−.34	−.24
Low prepotent sensible	−.05	.05	−.17	−.01
Sentence completion speed per item in seconds				
High prepotent nonsensical	.12	.17	.07	.14
Low prepotent nonsensical	.20	.16	.08	.20
Flanker percentage accuracy				
Compatible trials	.00	−.10	.05	.16
Incompatible trials	−.30	−.32	−.27	.07
Flanker speed per item in seconds				
Compatible trials	−.17	−.15	−.35	−.19
Incompatible trials	−.26	−.16	−.56*	−.34
N-back percentage accuracy				
0-back	−.18	−.28	−.43	−.37
1-back	−.31	−.27	−.26	−.36
2-back	.00	.08	−.13	−.35
N-back speed per item in seconds				
0-back	−.24	.02	−.07	.01
1-back	−.40	.01	.02	.10
2-back	−.02	−.08	−.19	−.41
Task switching percentage accuracy				
Switch trials	−.22	−.05	−.12	−.15
Nonswitch trials	−.30	.05	−.04	−.08
Task switching speed per item in seconds				
Switch trials	.30	.05	.19	−.15
Nonswitch trials	.24	.05	.02	−.41
Object alternation				
Percentage of trials correct	.02	−.01	−.20	−.09
Speed per item in seconds	−.34	.12	.14	−.05

Note. CAARS = Conners Adult ADHD Rating Scales—Short Version; LOI = Leyton Obsessional Inventory; MOCI = Maudsley Obsessive-Compulsive Inventory.
[a] $n = 19$.
* $p < .05$.

This showed significant correlations ($p < .05$) between Yale Tic Severity scores and sentence completion errors for both high and low prepotent sentences and between LOI obsessionality scores and Flanker speed on incompatible trials. There were no other significant correlations with the symptomatology measures. All significant correlations were in the expected direction, that is, higher symptomatology correlated with poorer performance.

Any effect of age on the results was examined by repeating the comparisons using age as a covariate. This did not alter the pattern of findings. The effects of medication on performance were examined within the TS group for the sentence completion measures that differentiated the groups. There was no significant difference between the medicated and unmedicated participants on these measures, nor any significant task by medication effects ($p > .05$).

Discussion

The findings showed that, as expected, the groups did not differ significantly in either accuracy or speed on measures of working memory, task switching, or object alternation learning. Predictions regarding inhibitory deficits were partially confirmed as the TS group made more errors than a matched control group on an inhibitory sentence completion measure. However, the interaction with prepotency was not significant, there were no group differences in speed of performance, and there was no difference on another inhibitory measure, the flanker task. There was little evidence that prescribed medication for the TS group influenced the findings, but the sample sizes were too small to draw firm conclusions about this.

To address issues relating to the influence of comorbid symptomatology, only TS participants who did not meet DSM criteria for comorbid disorders were included in the study. In addition, measures were included to assess comorbid symptomatology and tic severity, to examine the relationship between these and any executive deficits. The TS group differed significantly from the controls on the ADHD measure, but there were no significant correlations between ADHD scores and performance, suggesting that this was not a critical factor contributing to the pattern of findings. The TS group did not differ from the controls on the obsessionality measures, and there was a significant correlation between only one of these and a cognitive measure (inhibitory flanker speed), which did not differentiate the groups. Taken together, the findings suggest that comorbidity played at most a small role in any inhibitory difficulties shown by the TS group. With respect to Yale tic symptomatology, significant correlations were found with the one task that differentiated the two groups (sentence completion errors), suggesting that core TS severity was a relevant factor determining cognitive impairment.

What do the present findings imply for the inhibitory deficit hypothesis of TS? Sample size in the present study was relatively small, as is typically the case when studying a rare disorder, and it is possible given the number of tests performed that the significant comparisons arise simply by chance. Nevertheless, the findings are consistent with a number of previous studies in suggesting that any cognitive deficits associated with uncomplicated TS are relatively mild and circumscribed in nature.

The lack of impairment in the present TS participants on tasks involving working memory, task switching, and object alternation is in line with expectations. However, the inhibitory deficit hypothesis receives at best only partial support from the measures involving inhibition. The flanker task was expected to show impairment on incompatible but not compatible trials, but there was no evidence of group differences for either accuracy or speed. Performance on the sentence completion task was consistent with predictions based on an inhibitory deficit in that the TS group made more errors in completing sentences with nonsensical endings than the control group. However, the lack of group differences in speed and the lack of effect of prepotency of the nonsensical sentence completions did not provide support for the hypothesis.

Inhibition is not necessarily a unitary construct (see, e.g., Kok, 1999; Nigg, 2000), and the two tasks involving inhibition, sentence completion, and the flanker task differ in several ways. Any cognitive impairments in uncomplicated TS may be linked to very specific types of inhibition, and tasks involving inhibition of any response for a proportion of trials (such as the go/no-go or the stop signal paradigm) merit further study. The sentence completion task used in the present study involves the inhibition of prepotent responses (sensible completion words), whereas conflict on the flanker task involves the inhibition of interfering, but not prepotent, stimuli. Moreover, the sentence completion task also involves the use of strategic processes to search long-term memory stores for alternative responses. Crawford et al. (2005) suggested that higher error rates in sentence completion in children with uncomplicated TS might reflect the disruption of verbal or conceptual processes involved in task performance, rather than pure inhibitory processes. The TS participants may therefore have had difficulties developing efficient strategies such as those commonly used by normal participants, such as naming objects in the room or drawing words from semantic categories (Burgess & Shallice, 1996).

Several of the measures used in this study (sentence completion, flanker, *n*-back) are the same as those used by Crawford et al. (2005) to study children with TS. The findings of the two studies are comparable in showing higher error rates on the sentence completion task. However, in contrast with the earlier findings, adults with TS in the present study showed no evidence of slowed performance on the sentence completion task and also showed no evidence of differences on the flanker test. This suggests that any impairments associated with uncomplicated TS may be more pronounced, and hence easier to detect, in children than in adults. It may be that TS is associated with a lag in the development of certain cognitive skills and that maturation of these skills is slower but reaches normal or near-normal levels in adulthood. There may also be a direct relationship between cognitive impairments and the clinical picture often seen in TS, in which symptoms tend to diminish in frequency and/or severity with age, although other factors such as treatment response are also potentially relevant (for a discussion see, e.g., Robertson, 2000). Some tentative support for an association between TS symptomatology and cognitive function is provided by the significant correlations between sentence completion impairment measures and Yale tic severity scores found in the present study. However, longitudinal data would provide a more powerful test of any change over time in the relationship between TS symptomatology and cognitive functioning.

Overall, the present findings add to a growing body of evidence showing that uncomplicated TS is not associated with widespread executive impairments. The precise nature of performance deficits in both children and adults with TS merits further attention to determine whether these are essentially inhibitory in nature, and if

so, precisely which inhibitory processes are involved. There may be a range of separable inhibitory processes, both automatic and effortful, with different underlying neural substrates. The present findings also suggest that any impairment that does characterize uncomplicated TS may improve to some extent with age. Longitudinal studies are needed to address this issue more precisely, but it is certainly plausible that cognitive impairments detected in childhood become less marked in adulthood and may disappear for some individuals.

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